

with C. The amount of the inoculum injected in each case varied between 0.2 and 4.5 cc. In preparing the larger inoculums the sediment and liquid from several culture tubes were pooled to make up the requisite amounts for inoculation.

After an injection of pentobarbital sodium into the peritoneal cavity, the abdomen of the animal was opened through a high midline incision in the anterior wall. The pyloric end of the stomach was delivered through the wound. The anterior wall of the pyloric antrum or canal was chosen as the site for inoculation. The needle of the syringe was thrust through the serosa and muscularis, whereupon the required amount of inoculum was introduced into the submucosa. The accuracy of introducing the inoculum into the submucosa by such a blind method may be questioned, but the difference in the sense of resistance offered to the penetrating needle by the serosa and muscularis, on the one hand, and the submucosa, on the other, is so great that one is never in doubt regarding the position of the needle point. Moreover, it is almost impossible to enter the pyloric lumen with the needle for the reason that the mucosa bulges in advance of the needle point until it contacts the opposite wall, giving rise to the danger of penetrating the posterior wall rather than to that of entering the lumen. Owing to one accidental inoculation of the abdominal incision, special precaution against such contamination was thereafter strictly observed.

Altogether, 51 dogs and 8 young cats were inoculated. Only animals which looked healthy and well nourished were selected, in order that the results might be based on trustworthy material. The age of the first 4 cats ranged from 3 to 9 months, while that of the last 4 ranged between 7 weeks and 3 months. The first 14 dogs in the series were kept in individual cages. The other dogs were assigned to two fairly large rooms joined by a passage; here the dogs adapted themselves to confinement, played together and were maintained under a strict quarantine. The cats were kept in individual cages in a sunny room with good ventilation.

A milk diet was given during the first three to five days following inoculation and thereafter a general diet consisting of 1 part of fresh ground beef heart and 2 parts of a prepared food in a dry form, composed of cereal, bone, meat and minerals. It was found desirable to kill the animal to be examined in the morning when the stomach was empty. Immediately after death the cardiac and pyloric ends of the stomach were ligated, and the lumen was filled by injection of a 3.5 per cent solution of formaldehyde. The stomach was then removed and placed in a jar containing a solution of formaldehyde of the same strength. A careful autopsy was made on every animal in the series, either when it was killed or as soon as possible after death. Four hours later the stomach was opened along either the lesser or the greater curvature and carefully examined, after which the extent and intensity of the macroscopic lesion were recorded. Later the specimens were cut into small blocks of tissue, embedded in paraffin, sectioned and stained with hematoxylin and eosin; over 30 blocks of tissue were studied in serial section.¹⁶

OBSERVATIONS

The inoculation of human strains of *E. histolytica* into the gastric submucosa gave rise to the formation of gross gastric lesions in 36 dogs and 5 cats (69.49 per cent of the series); in the remaining 15 dogs and 3 cats (30.51 per cent) a lesion could not be demonstrated even with the

16. This work was carried out in the technic room of the department of anatomy under the direction of Dr. O. F. Kampmeier.

aid of a hand lens. The lesions were without exception confined to the stomach; the small and large intestine and the liver failed to show any gross evidence of extension. During the course of the experiment 21 dogs and 2 cats died; 6 dogs of this group presented, in addition to the gastric lesion, some intercurrent disease, while the remainder contained only the amebic lesion. At varying intervals after inoculation 30 dogs and 6 cats were killed. The duration of observation varied from one to one hundred and three days, the average being slightly over four days for the animals that died and about thirty-five days for those that were killed. The majority of the animals displayed only a moderate constitutional reaction to the inoculation; their appetite returned on the first or second day following the injection, and diarrhea or loss in weight was usually not observed with the infection. There were a number of animals that died while the amebic process was still in an early stage, 10 within twenty-hours and 8 within four days, and these showed a strong general reaction to the inoculation.

The earliest lesions observed in the series occurred in dogs that died within twenty-four hours. A lesion of such brief duration consisted of a region of hemorrhagic exudation in the submucosa with moderate congestion of the overlying mucosa; the involved layers presented considerable induration but no apparent thickening or elevation. Surrounding the area of exudation in the submucosa was a zone of infiltration consisting of lymphocytes, plasma cells, polymorphonuclear leukocytes and histiocytes. The gastric pits and glands were filled with a cellular débris, and many of the chief cells were detached from the basement membrane. There was marked engorgement of the involved capillaries and vessels. One dog of this group presented an hourglass constriction of the stomach at the level of the lesion, set up by tonic contraction of a segment of circular muscle fibers, and the hemorrhagic exudation in the lesion extended into the submucosa overlying the constriction.

Circumscribed nodules appeared at primary sites in dogs that died within three days after inoculation, and the various forms are displayed in figure 1. The extravasation of serum and red blood corpuscles into the interstices of the submucosa was sufficiently extensive to increase the depth of this layer to two or three times its normal thickness, and peripheral to it was a region of infiltration with numerous polyblasts, plasma cells and polymorphonuclear leukocytes. Many sections contained large depositions of hematin from the hemoglobin set free by disintegration of the red corpuscles. In some lesions the hemorrhagic exudation extended into the connective tissue septums of the inner part of the muscular coat, causing swelling of the septums and converting the adjoining smooth muscle tissue into a homogeneous hyaline material. The mucosa at the apex of the nodule was transformed into a mass

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FEBRUARY 1939

PUBLISHED MONTHLY BY AMERICAN MEDICAL ASSOCIATION, 535 NORTH
DEARBORN STREET, CHICAGO, ILLINOIS. ANNUAL SUBSCRIPTION, \$6.00

Entered as Second Class Matter Jan. 20, 1926, at the Postoffice at Chicago,
Under the Act of Congress of March 3, 1879

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PATHOLOGIC ASPECTS OF POSTERIOR PROTRUSIONS OF THE INTERVERTEBRAL DISKS

WALTER G. DEUCHER, M.D.

Fellow in Radiology, the Mayo Foundation

AND

J. GRAFTON LOVE, M.D.

ROCHESTER, MINN.

One hundred protruded intervertebral disks were studied. These had been removed by neurosurgeons of the Mayo Clinic from 94 patients operated on in the period from 1922 to 1937 because of compression of the cord and nerve root. True primary tumors of disks and tumors involving disks secondarily were not included in this report.¹ Each specimen was examined macroscopically immediately after its removal. The microscopic examination was done after fixation of the specimen in solution of formaldehyde U. S. P. (diluted 1 to 10) for at least twenty-four hours. The specimens, embedded in paraffin, were cut in the usual manner, and the sections were stained with hematoxylin and eosin as a routine. Because of their unevenness and hardness, the specimens were difficult to cut. In many cases several sections were made, representing various parts of the protrusion. In selected cases special stains were used: Mayer's mucicarmine stain, to reveal chondromucoid substance; Best's carmine stain, to show glycogen in notochordal cells, and Van Gieson's stain, to show fibrils of connective tissue.

The age distribution of the 94 patients (table) was not unusual for patients who have protrusion of an intervertebral disk. The average age was 38 years. Eighty per cent of the patients were men. The site of the protrusion likewise was not unusual. It is likely that the findings in this group of 100 persons with protrusion of an intervertebral disk will not differ from those in the larger group in which operation has been performed at the Mayo Clinic up to the present.²

From the Section on Neurosurgery, the Mayo Clinic.

1. Adson, A. W.; Kernohan, J. W., and Woltman, H. W.: *Arch. Neurol. & Psychiat.* **33**:247, 1935.

2. Love, J. G., and Walsh, M. N.: *J. A. M. A.* **111**:396, 1938.

MACROSCOPIC OBSERVATIONS

Grossly we can distinguish two different types of posterior protrusion of the intervertebral disk removed at operation. The tissue may be either in a single dense piece or in several fragments. These two forms of removed tissue correspond to the different types of protrusions³ which are found at operation. The characteristic appearance of the tissue removed in one piece is that of "wet rolled up blotting paper" or of "chewed up chalk." The size of the part removed in this series of cases ranged from that of a bean to that of a hickory nut (from 1 to 2.5 cm. in diameter). The size of the removed tissue did not correspond to that of the tumor in situ, because pieces which lay inside the normal area of the disk were removed with the protruded portion. The shape of the protrusion in situ is hemispheric or oval. The tissue loses this shape on removal; the fibrous elements which are compressed broaden out; in some of our cases it was possible to stretch the removed part to 8 cm. The tissue in general is firm, but there are usually small soft parts in it. The appearance of the various portions varies with the consistence of the tissue. The denser portions are made up of fibrous elements and frequently reveal a fine lamellar structure. One part of the border may be smooth for a short extent and the other marginal parts fringed. There are usually one or more pointed processes, which are smooth and shiny and do not look fibrous. These are the softest parts of the protruded tissue. In certain of our cases these processes had the appearance of little synovial fringes (fig. 1 A). At operation these processes were found to extend deep into the disk.

The other type of protrusion cannot be removed so easily, and it is necessary to cut or to use the rongeur to remove all of it. There may be adhesions to the disk or to surrounding structures, such as the vertebral body, the dura mater or the ligament. Such protrusions can seldom be removed in one piece. The removed mass consists of several fragments of different sizes and shapes. The number of fragments in the various cases in this series in which the tissue was in fragments varied from 2 to 11. The size of the various pieces varied accordingly. In some cases all of the pieces were about the same size; in others the largest piece was almost as large as the entire protrusion, and various small pieces made up the rest of the mass. The shape of the individual fragment therefore varies markedly and is meaningless, as it may be due to an artefact. Besides the variation of consistence mentioned previously, bony-hard parts are present in some fragments. These areas are small pieces of vertebra which have been removed with the protruded portion of the disk. The various fragments show the same differences as the tissue removed in one piece.

3. Love, J. G., and Camp, J. D.: *J. Bone & Joint Surg.* **35**:776, 1937.

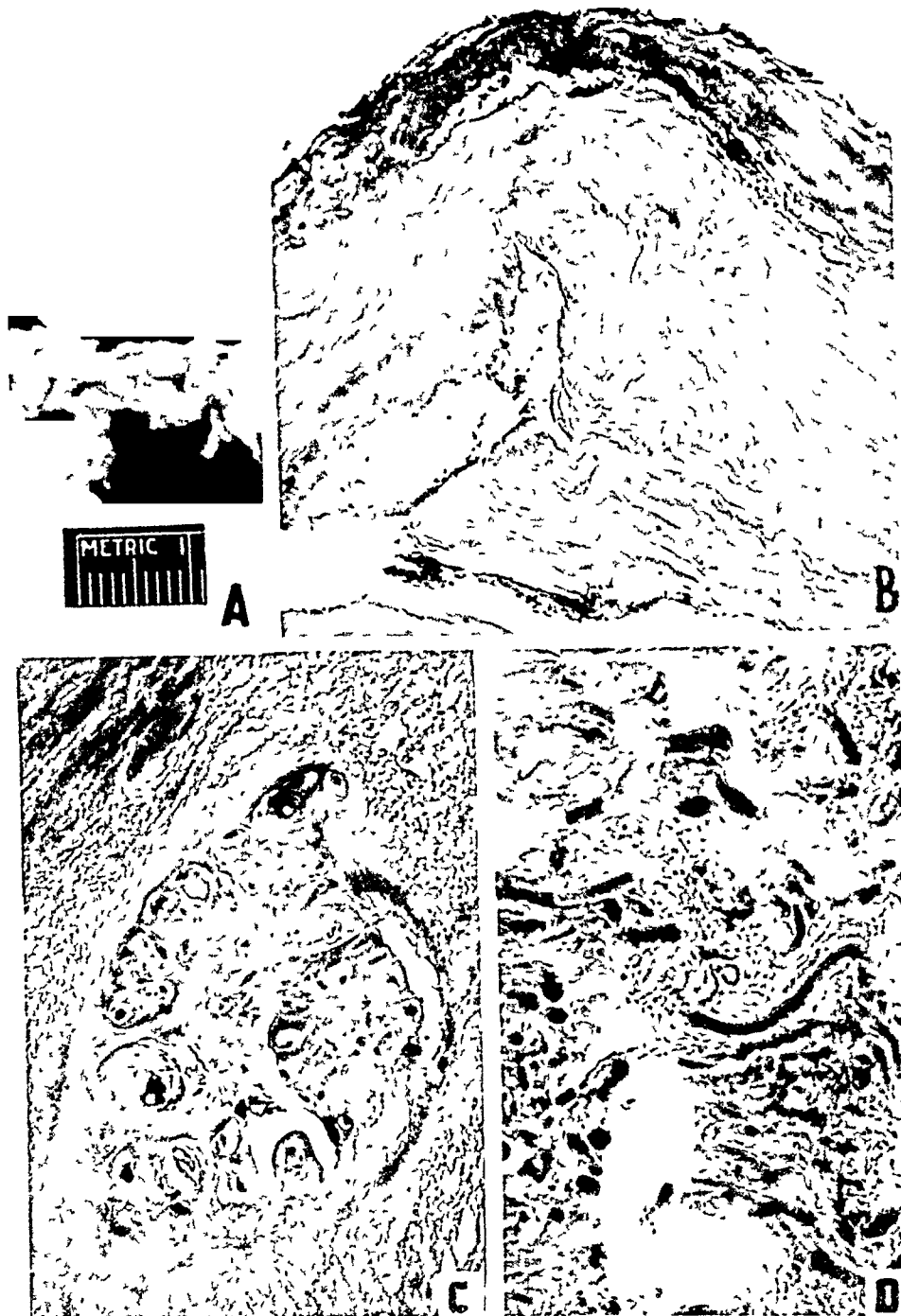


Fig. 1.—*A*, protruded portion of the fourth lumbar intervertebral disk, removed in one piece; the soft pointed processes with smooth, shiny surfaces extended into the central parts of the disk. *B*, bulging of the entire mass consisting of the outermost portion of the annulus, which is composed of parallel connective tissue fibers, and the underlying parts of the annulus; $\times 90$. The section is from the protruded portion of a lumbosacral disk of a man 33 years of age. The structural changes in the annulus consist of an irregular course of fibrils, fissures and unusually rounded cells. *C*, large island of cartilage cells in an advanced state of degeneration; the capsule of the cartilage is indistinct and partly broken; the cell outlines are vanishing; karyorrhexis is seen; $\times 240$. The section is from a protruded portion of the second lumbar disk of a woman 59 years of age. *D*, degenerated fibrils of an annulus; $\times 400$. The section shows short, swollen, broken-up and irregular fibrils and hyaline masses from a protruded portion of the fourth lumbar disk of a man 23 years of age.

MACROSCOPIC OBSERVATIONS

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The other type of protrusion cannot be removed so easily, and it is necessary to cut or to use the rongeur to remove all of it. There may be adhesions to the disk or to surrounding structures, such as the vertebral body, the dura mater or the ligament. Such protrusions can seldom be removed in one piece. The removed mass consists of several fragments of different sizes and shapes. The number of fragments in the various cases in this series in which the tissue was in fragments varied from 2 to 11. The size of the various pieces varied accordingly. In some cases all of the pieces were about the same size; in others the largest piece was almost as large as the entire protrusion, and various small pieces made up the rest of the mass. The shape of the individual fragment therefore varies markedly and is meaningless, as it may be due to an artefact. Besides the variation of consistence mentioned previously, bony-hard parts are present in some fragments. These areas are small pieces of vertebra which have been removed with the protruded portion of the disk. The various fragments show the same differences as the tissue removed in one piece.

3. Love, J. G., and Camp, J. D.: *J. Bone & Joint Surg.* **35**:776, 1937.

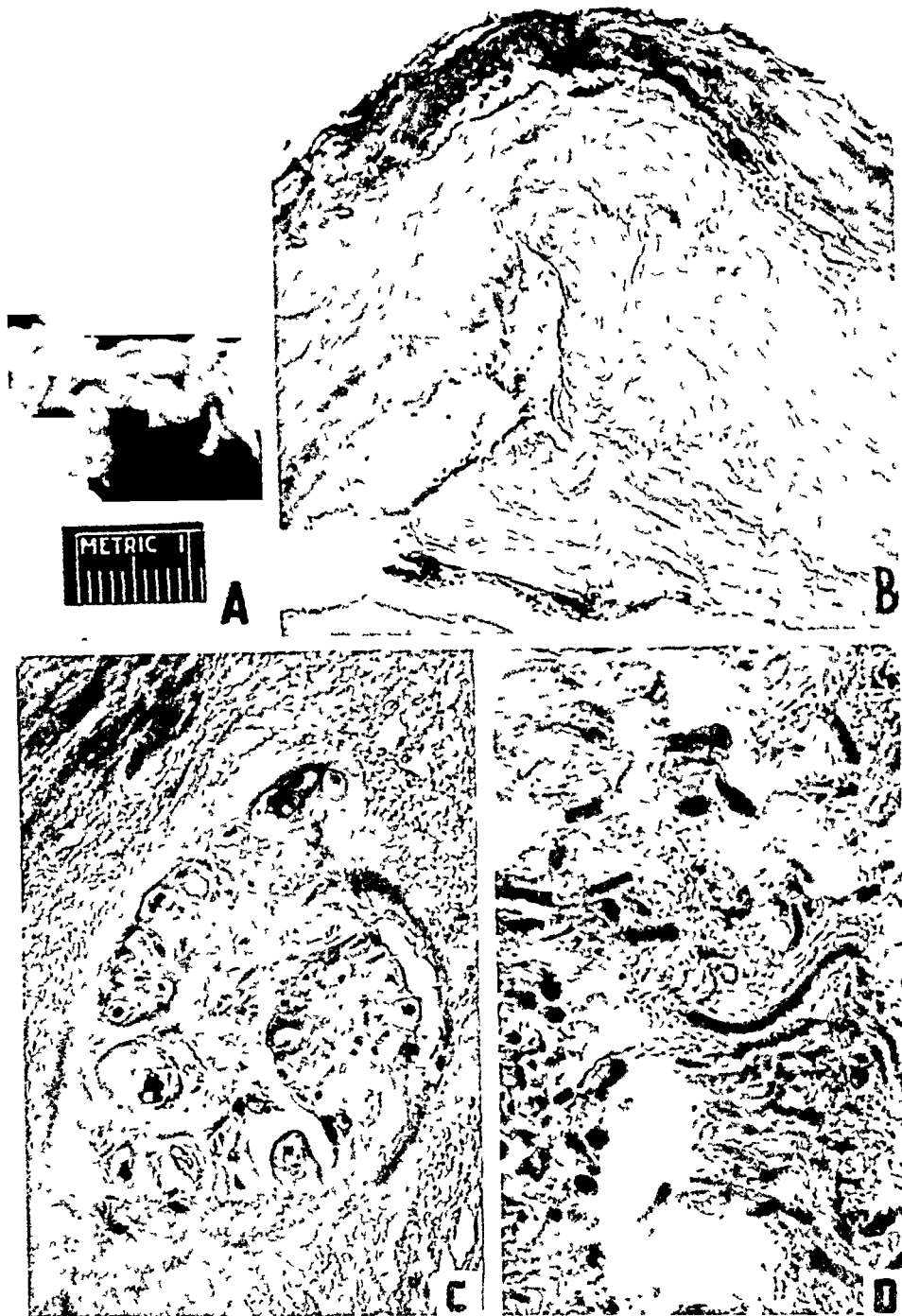


Fig. 1.—*A*, protruded portion of the fourth lumbar intervertebral disk, removed in one piece; the soft pointed processes with smooth, shiny surfaces extended into the central parts of the disk. *B*, bulging of the entire mass consisting of the outermost portion of the annulus, which is composed of parallel connective tissue fibers, and the underlying parts of the annulus; $\times 90$. The section is from the protruded portion of a lumbosacral disk of a man 33 years of age. The structural changes in the annulus consist of an irregular course of fibrils, fissures and unusually rounded cells. *C*, large island of cartilage cells in an advanced state of degeneration; the capsule of the cartilage is indistinct and partly broken; the cell outlines are vanishing; karyorrhexis is seen; $\times 240$. The section is from a protruded portion of the second lumbar disk of a woman 59 years of age. *D*, degenerated fibrils of an annulus; $\times 400$. The section shows short, swollen, broken-up and irregular fibrils and hyaline masses from a protruded portion of the fourth lumbar disk of a man 23 years of age.

It is evident from this description of protruded tissue that it is made of various portions of the disk and is not unaltered in all cases. Various authors⁴ have emphasized the fibrous character of the protrusion, but from this gross description no conclusion can be drawn about the changes which may have taken place in the tissue. As the outer portion of a normal disk is made of dense connective tissue, it is easy to understand the fibrous character of some of the fragments or pieces of a whole protrusion without assuming any radical pathologic change in the nucleus pulposus or fibrocartilaginous annulus fibrosus. In our material we found that frequently the marked fibrous character of separate pieces of a protrusion was caused by a relatively large admixture of the normal external portion of a disk. In the same way the separation of a protrusion into bits really has no microscopic significance.

MICROSCOPIC OBSERVATIONS

Our first interest lay in the composition of the protrusion that extended from the various portions of the normal disk. The intravertebral prolapses of the disk and the posterior protrusions which had not given rise to symptoms and which came to attention only at necropsy were usually made up chiefly, if not exclusively, of the nuclear portions of the disk.⁵ In our surgical material there was not a single specimen in which annular parts of the disk were not present also. The relationship of these two elements was variable; sometimes the annular and sometimes the nuclear portions predominated. This fact is of little consequence, as the line of demarcation between these two structures is indefinite. In many cases portions of the outermost annulus with its typical connective tissue fibrils and vessels were found. This finding disclosed that we were really dealing with a rupture of the annulus from bulging of the parts behind it. Only in a few cases were the outer portions of the annulus in close relationship to the lamellar elements just beneath it (fig. 1 *B*); more often this portion represented a separate fragment. Remnants of notochordal tissue were found in numerous cases. The notochordal cells were arranged in large or small irregular areas as a rule at the edge of the specimen or in fissures in the protrusion. Most of the notochordal cells stained positively for glycogen. Stains for mucus revealed this substance in some notochordal cells and showed it in great quantities around the cartilage cells of the nuclear portion.

4. Alajouanine, T., and Petit-Dutaillis, D.: *Presse méd.* **38**:1657 and 1749, 1930. Love, J. G.: *Proc. Staff Meet., Mayo Clin.* **11**:529, 1936. Mixter, W. J.: *Ann. Surg.* **106**:777, 1937.

5. (a) Andrae, R.: *Beitr. z. path. Anat. u. z. allg. Path.* **82**:464, 1929. (b) Beadle, O. A.: *The Intervertebral Discs: Observations on Their Normal and Morbid Anatomy in Relation to Certain Spinal Deformities*, Medical Research Council, Special Report Series, no. 161, London, His Majesty's Stationery Office, 1931.

Structural Changes.—In almost all cases the structure of the annulus fibrosus had undergone more or less definite change. The lamellae retained only for a short distance their normal regular course; then they broke off or followed a tortuous course. The cells of the annulus were often unusually round, and often abnormally large groups of cells were found. The changes in the course of the lamellae were probably a purely mechanical result of the bulging and rupture of the annulus. The change in the shape of the cells probably was due to changes in the functional stress on them.⁶ The forces active on the lamellar elements of the protruded disk must have been changed markedly, and these then produced the changes in the cells described through their long-continued effect. In the nuclear parts structural changes were less definite; the course of the fibrils remained normal, the cells, which indeed have no characteristic form, did not seem to be definitely changed except for the relatively frequent occurrence of large cartilage islands.⁷ In those areas the difference in the demands made on them was more quantitative than qualitative. Large and small irregular fissures were an almost constant finding in both the nuclear and the annular parts (fig. 1 B).

Degenerative Changes.—The normal disks of older men ordinarily show signs of marked degeneration.⁸ There are definite individual differences both in the time of appearance and in the degree of the changes. For this reason it is difficult to evaluate correctly the degenerative changes in a protrusion if, as in our purely operative material, it is impossible to draw comparisons with the rest of the involved disk or with other intervertebral disks of the same patient. In spite of this uncertainty the occurrence of degenerative changes was worthy of note. Degeneration of a slight or moderate degree could be found in practically all protrusions.⁹ Thirty-two of our 100 specimens showed advanced degeneration. These specimens were from patients in all the different age groups, and the percentage distribution was characteristic (table). Although degenerative changes appeared most frequently in the specimens from patients who were in the age group of from 51 to 60 years, marked degeneration of the protruded portion was not infrequent in specimens from young persons.

The degenerative changes involved the cartilage cells, the fibrils or both. Degeneration of the cartilage cells was much more frequent,

6. von Möllendorff, W.: *Handbuch der mikroskopischen Anatomie des Menschen*, Berlin, Julius Springer, 1930, vol. 2, pt. 2, pp. 40 and 225.

7. Elsberg, C. A.: *Bull. Neurol. Inst. New York* 1:350, 1931.

8. Schmorl, G., and Junghans, H.: *Fortschr. a. d. Geb. d. Röntgenstrahlen* 43:1, 1932.

9. Barr, J. S.; Hampton, A. O., and Mixter, W. J.: *J. A. M. A.* 109:1265, 1937. Love and Camp.³

being present in 27 cases. There occurred not only evanescent degenerative changes of single cells⁶ but also marked degeneration in islands of cartilage. The outlines of the separate cells in these islands of cartilage disappeared; the nuclei revealed karyorrhexis or pyknosis, and sometimes they had disappeared. All portions of the capsule of the cartilage were not intact; basophilic "halos" were sometimes extensive (fig. 1 C).

Degeneration of Fibrils.—This occurred usually with degenerative changes of the cells in the outermost portions of the annulus. The single fibrils were short, thick and swollen; some were broken off, some took an irregular course and some were coiled. Occasionally hyaline patches of varying size were found in which the fibrillar structure was no longer recognizable (fig. 1 D).

Fibrosis.—Fibrosis of the nuclear portion occurs frequently in normally placed disks. It represents the end stage of various pathologic changes and does not appear until blood vessels have invaded the field.⁸

Percentage Distribution of Patients at Time of Removal of Protruded Intervertebral Disks, Related to Percentage Distribution of Pathologic Changes and Presence of Notochordal Remnants in the Disk, in Age Groups

Age, Yr.	Cases	Pathologic Changes			Notochordal Remnants
		Degeneration	Edema	Fibrosis	
20-30.....	26	20	43	21	14
31-40.....	35	24	24	38	28
41-50.....	26	26	28	24	24
51-60.....	13	30	5	17	33
Specimens.....	100	32	22	28	28

Workers have remarked frequently that regressive fibrotic changes may take place in a protrusion of an intervertebral disk.⁴ In our material there was fibrosis of different grades in 28 specimens. In every one of these the nuclear portion of the protrusion showed definite changes, but only rarely was one encountered in which the annular portion showed fibrotic changes. In no instance was fibrous tissue completely substituted for the fibrocartilaginous structures of the intervertebral disk.

In 14 specimens the fibrosis was related to remnants of the notochord. At the edge of the specimen or along fissures in the tissue there were areas of varying extent of notochordal tissue. The cells of the notochord occasionally were degenerated. Near these cells and mixed up closely with them were connective tissue cells with delicate narrow dark nuclei, without any vacuoles. On the margins toward the fibrocartilaginous tissue the fibrous cells showed a marked tendency toward invasion. The margin between the two types of tissue was irregular and indefinite, and oftentimes connective tissue cells were scattered deeply in the cartilage. In cases of advanced fibrosis young capillary vessels were not rare. In cases in which fibrosis was most marked the edges and the fissures

in the tissue of the protrusion were lined by dense fibrous tissue, and in a few places small rests of notochordal cells were found. Near the fibrocartilaginous elements the fibrous tissue became thinner and thinner, and finally there were only scattered fibrocytes in an otherwise normal or degenerating fibrocartilage. The close relationship of the fibrosis to the rests of the notochord was most remarkable. Notochordal cells, according to Linck,¹⁰ pass through various stages during their life, and in the third stage they are very similar to connective tissue cells. In many of the previously described cases in our material we used the Van Gieson stain, which demonstrated plainly the presence of connective tissue fibrils in the fibrotic areas. We can say, therefore, that rests of the notochord in the protruded portion of the disk are often the point of entry for fibrous changes. Whether the fibrocytes come from the outside or are derived from the notochordal cells cannot be decided (fig. 2 A). This finding was made by Alpers, Grant and Yaskin,¹¹ also, but these authors regarded it as a transition of immature, undifferentiated cells into cells resembling cartilage cells in a tumor.

In 14 cases we found fibrosis which apparently had no relationship to notochordal rests. Foci of fibrocytes of varying size were present in otherwise normal or degenerating fibrocartilaginous tissue. At their edges these fibrous nests showed a tendency to invade the surrounding tissue. Their border was indistinct and irregular. The arrangement of the fibrils likewise was irregular. In the center of the nest the tissue was much denser, and it became looser toward the exterior, thereby giving the appearance of typical foci of fibrosis. The Van Gieson stain demonstrated connective tissue fibrils in great numbers. In cases in which these changes were advanced, young capillaries were present.

The portion of the protrusion which did not contain fibrotic changes was in some instances normal and in others markedly degenerated. Both types of fibrosis showed nothing characteristic if considered from the point of view of the various age groups (table). Whereas many fibrotic protrusions had to be removed at operation with a rongeur, others could be taken out in one piece without difficulty. In fact, some of them popped out.³

Edema.—In 22 cases there was definite edematous swelling of the protrusion. On viewing the sections of such protrusions the edematous character was plain to the naked eye because of pale staining with hematoxylin and eosin. On microscopic examination we found the stained elements widely separated. In the annulus the lamellar structure was plainly recognizable by this separation. In the nucleus pulposus the

10. Linck, A., quoted by Schwyzer, A.: *Minnesota Med.* **20**:15, 1937.

11. Alpers, B. J.; Grant, F. C., and Yaskin, J. C.: *Ann. Surg.* **97**:10, 1933.

interspaces of the network structure were wider. Cells were comparatively less numerous. In the annular portion they were markedly rounded. The nuclei seemed relatively small, and the protoplasm was completely clear and transparent except for a small rest. In the nuclear

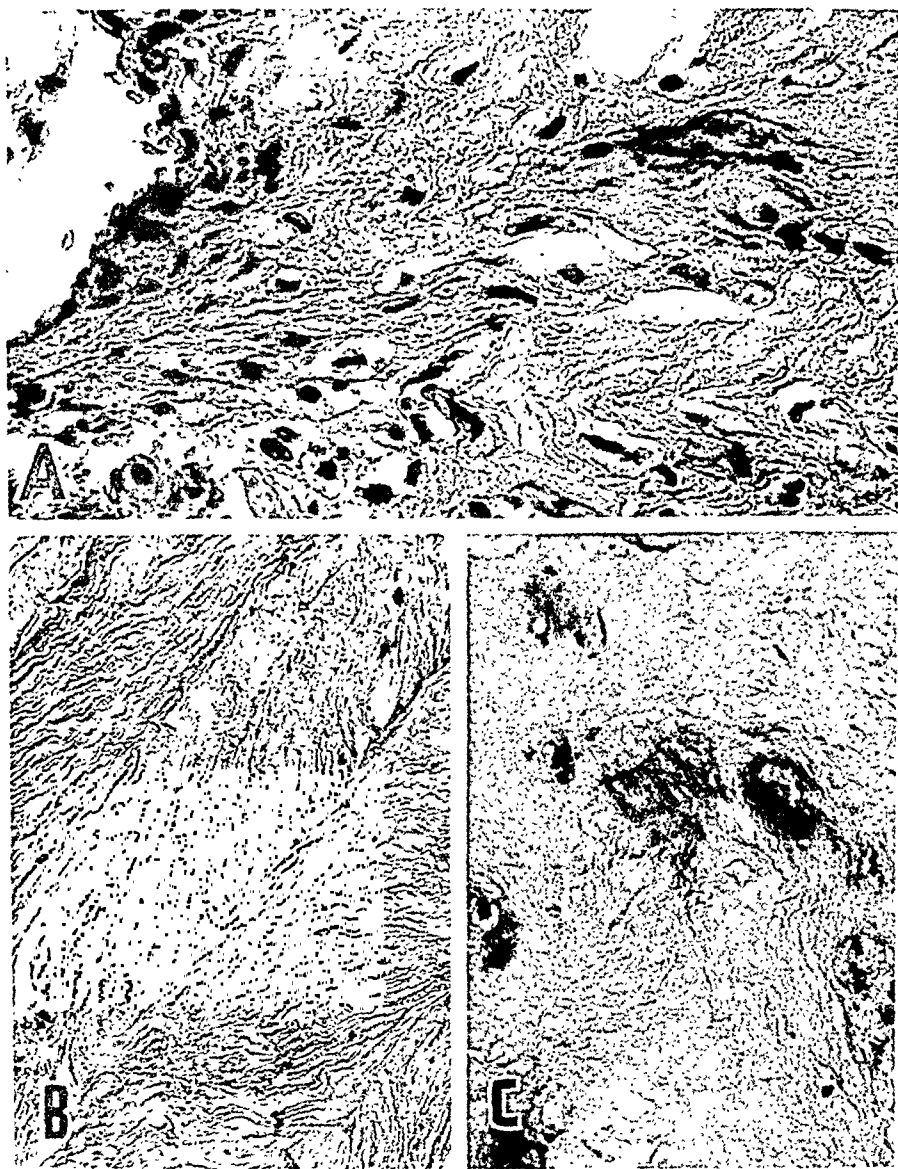


Fig. 2.—*A*, fibrosis and notochordal remnants of the protruded portion of the fourth lumbar disk of a woman aged 51 years; $\times 340$. Besides the typical physaliferous cells, the tissue shows connective tissue cells, a dense area at the edge of the fissure and an invasive tendency of the fibrous tissue. *B*, marked edema of the annulus fibrosus in a protruded portion of the fourth lumbar disk of a woman 26 years of age who had had recurrent symptoms for four years and whose last attack had begun six months prior to laminectomy; the fibrils are normal; the rounded cells contain clear protoplasm; $\times 40$. *C*, nuclear portion of a protrusion of the fourth lumbar disk of a man 32 years old who had had symptoms for three years and an exacerbation one month prior to laminectomy. Note the degenerating cartilage cells; $\times 200$.

part the edematous change in the cells was less marked. The fibrils of the annulus and nucleus took no part in the edematous swelling. They were unchanged, delicate and fine, but widely separated. Various sections through the tissue from one protrusion showed edema throughout, although it was not equally well marked in all portions. Edema of this type occurred in 11 cases, with marked degeneration of the cells (fig. 2 *B* and *C*). Little or no attention has been paid previously to this point. In our material marked edema was a frequent occurrence, and slight edema was present in the majority of cases. The age of the patients from whom the specimens exhibiting marked edema had been removed was significant (table). In 20 of our 22 cases attacks of pain lasting for from three weeks to six months had occurred immediately before registration at the clinic. Attacks of varying degree prior to these had ceased. In the other 2 cases the history was somewhat indefinite, but there had been symptoms for three and two years, respectively. These facts lead to the supposition that edema may be the cause of the recurrent attacks or exacerbations of pain. In many of the cases in which repeated painful attacks occurred over a period of years, a relatively small protrusion was found at operation, so that it seems likely that in some cases at least the repeated attacks were the result of recurring edema of the protruded portion of the disk. Edematous swelling and its subsidence under altered conditions, such as rest in bed, seem to offer one explanation of the intermittency of pain in these cases.

The marked capacity of the intervertebral disk, particularly of the nucleus, of the young person to swell is well known and definite.⁸ It has been demonstrated that the disk can swell to twenty times its normal size when placed in water.¹² The nucleus is kept to its normal size by the various forces acting on it from all sides. If these forces are decreased, the nucleus swells by absorption of fluid.¹³ In a case of protrusion the resistance to expansion of the protruded portion is naturally decreased, and swelling of the nuclear element, provided its capacity to swell is unaltered, is to be expected. In our material we saw swelling not only in the nuclear parts but also in parts of the protruded annulus. It is therefore very likely that displacement of a portion of the intervertebral disk results in circulatory changes favoring the development of edema which will involve the nucleus and the annulus. After a time this edema may recede and may recur later if opportunity is offered. Accidental changes in the position of the protrusion relative to its surroundings as a result of activity of the patient may represent such an opportunity. The edema seems therefore to be

12. von Puky, P.: *Arch. f. klin. Chir.* **188**:171, 1937.

13. Beadle.^{5b} Schmorl and Junghanns.⁸

the effect of the natural tendency of the nucleus to absorb fluid and of the circulatory changes resulting from the displacement of the tissue.

Such a process reminds us of the result of Ribbert's¹⁴ early experiments. After drilling or cutting a hole in the anterior part of the disk he saw small "tumors" develop, which increased in size for about fifty-two days and then stayed about the same size or, as Leopold and Zahn believed, decreased again. Ribbert called attention to the occurrence of large vacuoles in the cells, which were largest when the swelling had reached its maximum. A gradually increasing and then receding edematous swelling of the protruded parts could easily explain these findings and would be in accord with the observations made on our material.

We have mentioned that edema of the protrusion may be of clinical significance. This edema also may account for the well known discrepancy between the age distribution of the patients who have had clinical symptoms from protruded intervertebral disks and who have undergone operation and that of persons whose protrusions have been observed incidentally at necropsy. In old persons the capacity of the nucleus pulposus to swell is markedly decreased owing to the frequent degenerated, dried, fibrotic or crushed character of this part.⁸ Protrusions of the intervertebral disks in such persons have, therefore, much less tendency to become edematous, and for this reason they probably produce no symptoms.

SUMMARY

Posterior protrusions of the intervertebral disk causing symptoms which lead to operation are composed of all parts normally found in the unprotruded intervertebral disks, the annulus lamellosus, including its outer parts, and the nucleus pulposus with its occasional remnants of the notochord. The tissue of the intervertebral disk is almost invariably altered in the protrusion. The most common and constant changes in it consist of alterations of the normal architecture.

Degenerative changes, too, are seen commonly. Advanced degeneration is more frequent among patients in the older age groups. Marked degeneration of the cartilage cells is much more common than degeneration of the fibrils.

Fibrosis may occur in the form of proliferating fibrous tissue or it may be in close relationship to remnants of the notochord. In both instances the fibrous tissue tends to replace the normal fibrocartilaginous structures of the protruded portion of the disk.

Edema of the protruded part of the intervertebral disk is a most important and frequent finding. It may involve the annulus as well as

14. Ribbert: *Verhandl. d. Cong. f. inn. Med.* **13**:455, 1895.

the nucleus and is more frequent in young persons. The edema of the protrusion must be considered as a result of the capacity of the nucleus to swell if the normal forces keeping it in place and shape are decreased. At the same time it may be helped or held back by circulatory changes resulting from the displacement of the protruded part. The occurrence of edema may result in exacerbation of the clinical symptoms. Such an exacerbation, however, may subside spontaneously or with conservative treatment.

From this study it is not possible to draw any conclusions about an etiologic relationship of notochordal rests to protrusions of intervertebral disks.

PHAGOCYTOSIS OF TRYPAN BLUE IN RATS OF DIFFERENT AGE GROUPS

JAMES T. CULBERTSON, PH.D.

NEW YORK

It is generally recognized that man and animals manifest, as they grow older, increasing natural resistance against many kinds of infectious agents.¹ It appears likely, in spite of strong evidence to the contrary in a number of diseases, that this gradually developed natural resistance is often largely a matter of constitutional maturation and that exposure to a specific disease agent is not essential in order that man or animals may acquire resistance with age. As yet, the precise cause of the greater resistance of older animals is obscure, although much experimentation has been done on the question. It has long been known, for example, that older animals respond more powerfully in the production of serum antibodies than do younger ones after parenteral administration of antigenic substances. A difference in response analogous to that to an artificial stimulation might be expected after exposure to a natural infecting agent. Jungeblut and Engle² found experimentally that hormonal factors are of significance in the natural acquisition of resistance. Indeed, in discussing the naturally acquired resistance of adult persons against poliomyelitis, they^{2a} stated “. . . epidemiologic observations and experimental facts can be reconciled with a point of view emphasizing the normal endocrine balance characteristic of mature age as a major source of this protection.”³

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

1. The literature has been reviewed comprehensively by G. P. Ssacharoff (*Ergebn. d. allg. Path. u. path. Anat.* [pt. 2] **22**:201, 1927-1928) and by L. Baumgartner (*Yale J. Biol. & Med.* **6**:403, 1934).

2. Jungeblut, C. W., and Engle, E. T.: (a) *J. A. M. A.* **99**:2091, 1932; (b) *J. Exper. Med.* **59**:43, 1934.

3. Other mechanisms to explain observed differences in the resistance of animals of different age groups to infectious agents have been suggested. A. O. Foster (*Am. J. Hyg.* **24**:109, 1936) expressed the belief that age resistance is linked with the natural age curve of the hemoglobin level of a given host, especially since he was able to note an inverse correlation between the resistance of dogs and cats to hookworm infection and the anemia occasioned by hemorrhage or by feeding a milk diet, which is deficient in iron. A. O. Foster and W. W. Cort (*ibid.* **21**:302, 1935) found that young dogs are more susceptible than older dogs to the effects of a deficient diet in decreasing the natural resistance to *Ancylostoma caninum*.

It seems likely that the endocrine balance of an animal, as well as its improved capacity with age to produce specific antagonistic substances, is of fundamental importance in the natural acquisition of resistance. But it seems probable as well that the enhanced resistance of the older animals is in part dependent on a more effective functioning of the phagocytic cells of the older body, especially since, as Gay^{3a} pointed out, "body cells, rather than body fluids, are the first and the last factors in the defense processes against micro-organisms."

There is a considerable literature indicating that significant changes do occur in the reactive capacity of the defense cells as animals mature. For example, negative or only slight reactions are noted in the skin of infants given injections of such nonantigenic substances as turpentine, iodoform, mustard oil and other cutaneous irritants, whereas the skin of older persons reacts strongly to these substances (Adelsberger;⁴ Tachau^{4a}). Similarly, the skin of young infants does not react to large injections of diphtheria or scarlet fever toxin, even in the absence of demonstrable antitoxin (Schick test and Dick test), although the skin of somewhat older children does respond (von Gröer and Kassowitz⁵). Furthermore, Sarles⁶ has made the significant observation that old dogs experience a severe skin reaction, with prolonged inflammation, to the penetration of the dog hookworm, *Ancylostoma canium*, in contrast to the slight, transient effects in young dogs. The larvae of the hookworm are retained in the skin longer in old dogs than in young ones, and sections reveal partial disintegration of these parasites in the older animals.

An observation by Duca⁷ in this laboratory has thrown further light on the increasing reactivity of the protective cells as animals become older. Duca noted that nursling rats, which are frequently killed by *Trypanosomia lewisi*, show no change either in the total number of leukocytes or in the monocyte percentage after infection, whereas older rats, beginning very soon after being weaned, manifest a sharp rise in both the total leukocyte count and the monocyte percentage after infection, these responses being apparently related to the resistance which the older animals exhibit against *T. lewisi*. This observation has seemed to me to be of considerable significance and has led me to investigate further the general problem of naturally acquired resistance, especially with the purpose to determine the role played by the phagocytic cells in this resistance.

3a. Gay, F. P.: Arch. Path. **1**:590, 1926.

4. Adelsberger, L.: Ztschr. f. Kinderh. **43**:373, 1927.

4a. Tachau, P.: Ztschr. f. Kinderh. **38**:638, 1924.

5. von Gröer, F., and Kassowitz, K.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **30**:154, 1920.

6. Sarles, M. P.: Am. J. Hyg. **10**:683, 1929.

7. Duca, C. J.: Am. Heart J., to be published.

The present paper offers the results of an attempt to determine the relative capacity of the cells of rats of different age groups to phagocytose inert particles injected into these animals. It was hoped to find through the use of trypan blue whether the phagocytic function of these cells becomes more effective as the animals approach maturity. If such an improvement in function was found it was considered these same cells would be shown, by analogy, responsible in part for the enhanced resistance which older animals manifest against living infectious agents.

METHODS

Suspension of Trypan Blue.—Trypan blue was prepared for injection by suspending the dry powder in distilled water, in a concentration of 1 per cent. The suspension was sterilized in the autoclave prior to being injected.

Strain of Rat Used.—All rats were of the Sherman strain and were propagated in the Department of Animal Care of the College of Physicians and Surgeons. They were maintained on a diet consisting of the following foods in the percentages given: hulled oats (15), whole wheat (15), yellow corn (15), shelled barley (15), soybean meal (15), powdered whole milk (10), commercial dried meat scraps (Swift's) (10), green alfalfa meal (2), sodium chloride (2) and calcium carbonate (0.5).

Experimental Procedure.—Rats of successive age groups to maturity were used in this experiment: 7 were 6 days old, 5 were 10, 3 were 15, 4 were 18, 3 were 23, 3 were 40, and 2 were 60 days old. Half of these animals received by intraperitoneal injection 0.1 cc. of the suspension of trypan blue per 10 Gm. of body weight. The remainder were given twice this dose. Eighteen hours later, all of the rats were killed with ether, and pieces of liver, spleen, lung, brain, cartilage (xiphoid process) and skin removed and placed in Bouin's⁸ fixative. The tissues were embedded in paraffin, sectioned and stained lightly with eosin only.

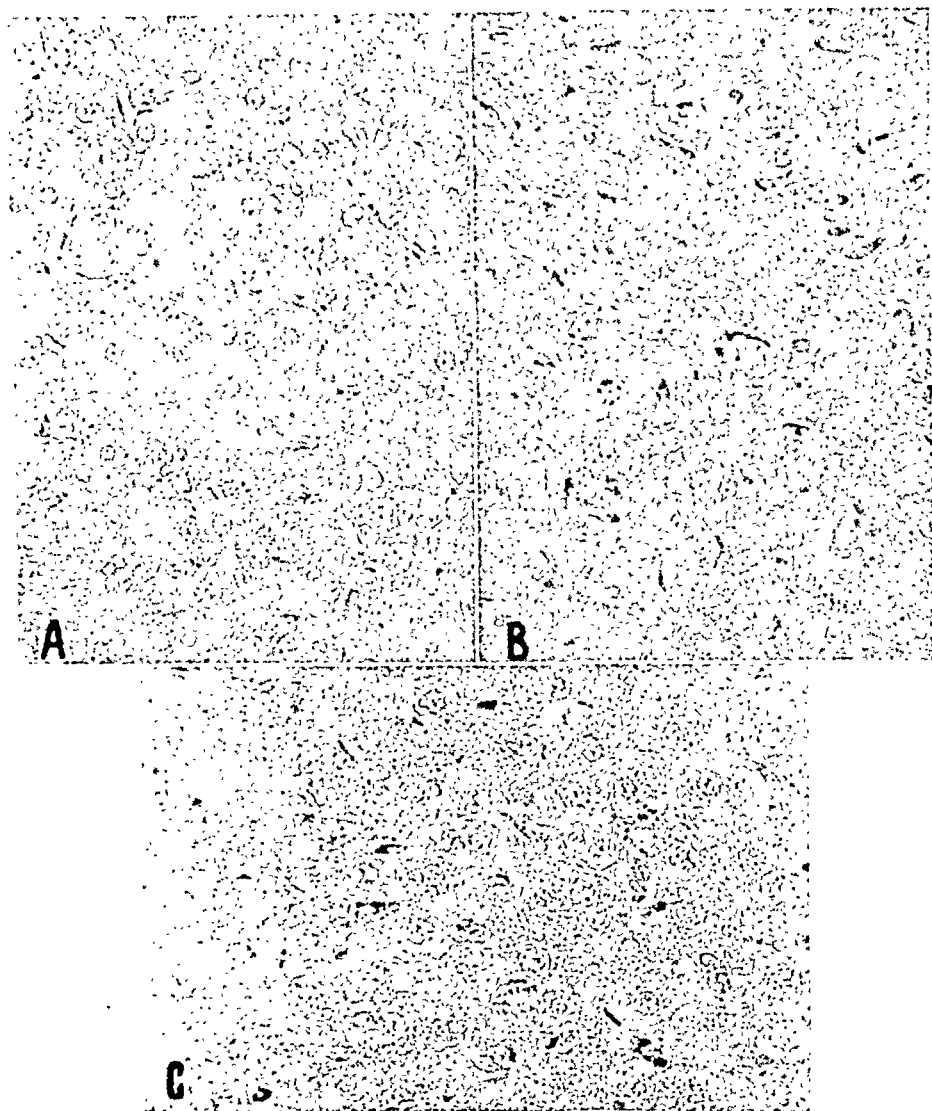
OBSERVATIONS

The examination of the prepared sections revealed no significant difference in the phagocytic capacity of the cells of any of the organs of the various age groups except the liver. In the liver, however, well marked and consistent differences were observed. Not only did more Kupffer cells in the livers of the older rats contain particles of the dye, but larger numbers of particles were to be found within each phagocytosing Kupffer cell of the older animals. The observed difference is fairly well brought out in the figure, in *A*, *B* and *C*.

An attempt was made to estimate quantitatively the difference seen in the rats of different age groups. The total number of Kupffer cells which contained any trypan blue particles whatsoever was determined in 25 representative oil immersion fields of each slide, the oil immersion objective being used in order to recognize unmistakably the individual

8. The formula is: Saturated aqueous solution of trinitrophenol, 75 parts; solution of formaldehyde U. S. P., 25 parts; glacial acetic acid, 5 parts.

particles of the dye. The quantitative results substantiated the impression given by a more superficial perusal of the slides, smaller numbers of cells, with fewer particles per cell, being observed in the animals less than 18 days old than in those over this age. It appeared, furthermore, that by the time an animal had reached 18 days of age its Kupffer



Sections of the livers of rats of different age which had received injections of trypan blue. They show the relative amount of phagocytosis by Kupffer cells. Stain, eosine only; magnification, about $\times 500$. *A* represents rat 10, 10 days old; *B*, rat 17, 18 days old, and *C*, rat 26, 60 days old.

cells had attained their full capacity for phagocytosis, since with further development rats showed little or no continued improvement in phagocytic potentialities. The results of the quantitative study, together with data relevant to each animal used, are given in the table.

Phagocytosis of Trypan Blue by the Kupffer Cells of Rats of Different Age Groups

Rat	Age, Days	Weight, Gm.	Amount of 1% Suspension of Trypan Blue Injected Intraperitoneally, Cc.	Kupffer Cells Showing Engulfed Particles in 25 "Oil Immersion" Fields ($\times 930$) of Section
1	6	11.0	0.11	20
2	6	11.5	0.115	1
3	6	11.5	0.115	44
4	6	12.0	0.12	31
5	6	12.5	0.125	0
6	6	11.5	0.23	5
7	6	12.5	0.25	0
8	10	12.0	0.12	0
9	10	12.0	0.12	40
10	10	12.0	0.12	89
11	10	11.5	0.23	102
12	10	12.0	0.24	156
13	15	17.0	0.17	174
14	15	19.0	0.19	112
15	15	19.0	0.38	186
16	18	25.0	0.25	260
17	18	27.0	0.27	280
18	18	24.0	0.48	217
19	18	25.0	0.50	313
20	23	40.0	0.40	268
21	23	50.0	0.50	279
22	23	39.0	0.78	348
23	40	102.0	1.02	265
24	40	105.0	1.05	302
25	40	84.0	1.68	278
26	60	88.0	0.88	264
27	60	87.0	1.74	284

COMMENT

The observations recorded in this paper show clearly that at birth rats are not provided with so effective a means of phagocytosing particles of trypan blue parenterally introduced as they later acquire. This difference has been demonstrated thus far only with the Kupffer cells of the liver, but it appears likely that other phagocytosing cells may show a similar nonspecific difference in function with age, though perhaps in lesser degree. At any rate, since the Kupffer cells have long been shown to be among the most significant cells of the defense mechanism, the demonstration of this difference with them alone goes far toward implicating an improvement in function with age in phagocytic cells as an important force in natural resistance. At the same time, this single defense agency must not be considered solely responsible for natural resistance. The gradually acquired capacity of animals to elaborate antibodies as well as hormones, the changes in the permeability of tissues in general and the alterations in the natural diet as animals grow older, together with differences in genetic constitution and other fundamental differences between animals, must be considered in attempting to explain the natural resistance acquired with maturity.

CONCLUSIONS

There is a difference in capacity for phagocytic function between young and old rats, the Kupffer cells of nursing animals being less able to phagocytose particles of trypan blue than the Kupffer cells of older animals. This difference can be correlated with a gradually acquired resistance of rats against a natural blood flagellate (*Trypanosoma lewisi*) of this animal. It is suggested that the resistance which the rat naturally acquires against this parasite as it grows older is in part accounted for by a gradually acquired enhancement in the phagocytic capacity of the host's cells.

ENDAMOEBA HISTOLYTICA

EXPERIMENTAL INFECTION OF THE STOMACHS OF DOGS AND CATS

YNGVE JORANSON, M.D.

AND

JOSEPH SIMONS, M.D.

CHICAGO

The object of this investigation was to induce infection with *Endamoeba histolytica* in the gastric submucosa of dogs and cats and study the nature of the tissue reaction. The animals were infected by inoculating the submucosa with cultured forms of *E. histolytica* of human origin. As long as the overlying mucosa remained intact, there was reason to believe that the lesion arising at the seat of inoculation would resemble the basic lesion of amebic disease. But with the disintegration of the mucosa and the consequent exposure of the amebas to the action of the gastric juice, the fate of the trophozoites and the further development of the lesion became a matter of speculation.

It has long been thought that gastric juice exerts a destructive action on the trophozoites of *E. histolytica*. Councilman and Lafleur¹ reported that *E. histolytica* exerts no action on the stomach because the conditions there are unsuitable for its multiplication. Ujihara² incubated cysts of *E. histolytica* with gastric juice for twenty-four hours at 37 C. and found that the greater part remained undigested. Penfold, Woodcock and Drew³ used pepsin in an acid medium as an excysting agent and obtained negative results. Chatton⁴ fed cysts to cats and killed the animals after periods varying from three and one-half to seventeen hours; except for the disappearance of the chromidial bars, he found that the cysts passed through the stomach unchanged.

The resistance which the cysts of *E. histolytica* display toward the action of hydrochloric acid has also been investigated. Yorke and

From the Department of Anatomy and Department of Bacteriology and Public Health, College of Medicine, University of Illinois, and the Research Laboratories of the Illinois Department of Public Health.

1. Councilman, W. T., and Lafleur, H. A.: Johns Hopkins Hosp. Rep. **2**:395, 1891.

2. Ujihara, K.: Ztschr. f. Hyg. u. Infektionskr. **77**:329, 1914.

3. Penfold, W. J.; Woodcock, H. M., and Drew, A. H.: Brit. M. J. **1**:714, 1916.

4. Chatton, E.: Bull. Soc. path. exot. **10**:834, 1917.

Adams⁵ exposed cysts to hydrochloric acid in various dilutions and at varying temperatures for thirty minutes. The exposed cysts were then cultured. From cysts exposed to 0.2, 1 and 5 per cent hydrochloric acid at from 20 to 25 C. they obtained positive sixteen hour old cultures of vegetative amebas. Dobell and Laidlaw⁶ employed the same acid in a constant dilution at a constant temperature and found that the cysts of *E. histolytica* readily withstood 0.2 per cent hydrochloric acid for two hours at room temperature and that some cysts hatched after even three hours' exposure.

Hegner⁷ believed that the fate of the trophozoite of *E. histolytica* after exposure to hydrochloric acid might have some bearing on the biologic significance of encystment, and injected trophozoites from carefully prepared cultures of *E. histolytica* into the stomach of a guinea pig. The animal was killed one hour later, but no amebas were present in the stomach; specimens, alive and moving, were recovered in the small intestine 6, 12, 20, 28, 34, 38, 44 and 51 inches (15, 30.5, 50.5, 71, 86, 96.5, 111.5 and 129.5 cm.) distal to the stomach. From this result Hegner concluded "that trophozoites may pass unharmed through the anterior portion of the alimentary canal and apparently may set up infections in the regions where they are normally localized, but that this probably is not the usual method of infection in nature."

Dobell⁸ also studied the effect of acidity on the trophozoites of *E. histolytica*. He exercised the greatest precaution in obtaining material which contained only unencysted forms. His experimental results indicate that the trophozoites can withstand exposure to 0.18 per cent hydrochloric acid at 37 C. for any time up to one full hour. Dobell stated that "in the acid the amoebae at once become rounded and motionless, and most of them soon appear—and are—dead or dying. It is only by attempting to make cultures from them afterwards that one can determine whether such forms are really dead or alive."

The greatest precaution should be observed in translating the results of these experiments into terms of amebic infection in man. The demonstration of the acid resistance of *E. histolytica*, particularly in its unencysted forms, raises the question whether the trophozoites live as harmless commensals within the lumen of the stomach or as parasites able at intervals to invade the gastric mucosa. In any consideration of host-parasite relations it is necessary to take into account the resistance of the host as well as the virulence of the parasite. The ability of the

5. Yorke, W., and Adams, A. R. D.: *Ann. Trop. Med.* **20**:279 and 317, 1926.

6. Dobell, C., and Laidlaw, P. P.: *Parasitology* **18**:283, 1926.

7. Hegner, R. W.: *Am. J. Hyg.* **6**:593, 1926.

8. Dobell, C.: *Parasitology* **19**:288, 1927.

gastric epithelium to resist both the mechanical penetration and the lytic action of *E. histolytica* is an important factor. But such resistance does not follow immutable standards. The gastric mucosa is constantly being exposed to mechanical and thermal injury, and the normal capacities of its epithelium for resistance against infection may thus be modified. If, nevertheless, the possibility of trauma is omitted from consideration, it may still be open to question whether the hydrochloric acid is able at all times and in all parts of the stomach to prevent an invasion of the normal mucosa by *E. histolytica*. From a theoretic standpoint it should not matter whether the organism invades the epithelium of the colon or of the stomach except as the location may affect the distribution of the endoparasite; once past this epithelial barrier the amebas are in position to initiate a lesion. But the probability of an invasion of the mucosa in the human stomach by *E. histolytica* is reduced to one of great uncertainty when it is borne in mind that such an involvement would hardly pass unnoticed in the histologic study of gastric lesions.

The human stomach has never been reported as the seat of a primary lesion in amebiasis; it has been mentioned by several investigators as a site for complications. Craig⁹ at autopsies examined 60 persons who died of amebic dysentery with special reference to complications and observed that the stomach was chronically inflamed in 1 of almost every 2 examined. The form of gastritis noted came next in frequency to chronic enteritis and was sufficiently severe to be a factor against recovery. Clark¹⁰ studied the reports of postmortem examinations in 186 cases of amebiasis and cites 2 cases in which an abscess of the liver involved the stomach, causing in one case an obstruction and in the other a perforation of the stomach. James¹¹ reviewed the findings of Clark and also examined the protocols of the observations at autopsy in 29 additional cases of amebiasis. He noted that there is no a priori reason why amebas that have passed the liver should not lodge in any or all of the organs or localities in which the parasite has been claimed to be present.

But if this were true [said James] some indication of such localization might reasonably be expected at those autopsies, in which all the damage possible appears to have been done. When one sees in a single instance the entire colon a mass of ulcers, from the ileocecal valve to the rectum, the liver riddled with multiple ulcers, without any other evidence of amoebic invasion, one is permitted a certain scepticism as to whether this parasite is often carried to other localities, or is the cause of not infrequent pathological conditions elsewhere.

9. Craig, C. F.: *Am. J. M. Sc.* **128**:145, 1904.

10. Clark, H. C.: *Am. J. Trop. Med.* **5**:157, 1925.

11. James, W. M.: *Ann. Trop. Med.* **22**:201, 1928.

The records of over 20,000 amebic patients and the reports of over 3,000 autopsies on patients with amebiasis were reviewed by Musgrave¹² in his twenty years of tropical experience. The article embodying the results of this extensive clinical study was edited by Reed, who wrote thus concerning complications:

Stomach disorders are particularly frequent in all tropical countries and naturally are often associated with amoebic disease in a casual manner. However, in addition to this, there is more frequent and undoubtedly mutually dependent association between the two conditions, particularly in the later stages of a prolonged amoebic infection. Gastralgia is particularly frequent and may be present with but mild amoebic infection. The different types of gastralgia with achylia gastrica and hyperchlorhydria are seen and gastric ulcer is relatively more frequent than in other diseases. After eliminating the usual factors, there remains frequent association of the two diseases which is hard to explain without acknowledging some form of interdependence.

It seems that inoculation of the gastric submucosa with cultured forms of human strains of *E. histolytica* and observations on the development and nature of the resulting lesions might add something to present knowledge of the characteristics of *E. histolytica* and perhaps throw some light on the causative factors underlying the process of ulceration in the human stomach.

MATERIALS AND METHODS

Three different strains of *E. histolytica* of human origin, identified separately by the letters C, G and H, were used in these experiments.¹³ The amebas were maintained in artificial cultivation on slants of liver infusion-agar overlaid with sterile rice flour.¹⁴ Every forty-eight hours transplants were made from sediment obtained at the bottom of the culture tube by means of a pipet and incubated at 37 C.¹⁵ Every strain grew well in this medium, and the development of the amebas in vitro was consistent for each strain. The material for injection contained the amebas and an accompanying flora of yeasts and bacteria.

The viability of the inoculum was tested prior to inoculation by placing a drop on a glass slide, adding a drop of 5 per cent aqueous solution of eosin and studying the microscopic details; all specimens contained at least from 10 to 15 amebas per field, and in some the organisms were much more numerous. The inoculums employed in two thirds of the experimental animals of the series contained only strain G, those in 5 animals contained only strain H, while those for the rest of the animals consisted of a combination of G with H or of H

12. Musgrave, W. E.: *Am. J. Trop. Med.* **11**:469, 1931.

13. The strains were obtained in culture from Dr. Bertha Kaplan Spector (deceased), associate protozoologist, United States Public Health Service, Chicago, and research associate in the department of medicine of the Douglas Smith Foundation of the University of Chicago, who originally cultivated them from patients infected with *E. histolytica*.

14. Cleveland, L. R., and Collier, J.: *Am. J. Hyg.* **12**:606, 1930.

15. The strains were cultivated in vitro by Miss Virginia Ryan, research bacteriologist in the research laboratories of the Illinois Department of Public Health, Chicago.

with C. The amount of the inoculum injected in each case varied between 0.2 and 4.5 cc. In preparing the larger inoculums the sediment and liquid from several culture tubes were pooled to make up the requisite amounts for inoculation.

After an injection of pentobarbital sodium into the peritoneal cavity, the abdomen of the animal was opened through a high midline incision in the anterior wall. The pyloric end of the stomach was delivered through the wound. The anterior wall of the pyloric antrum or canal was chosen as the site for inoculation. The needle of the syringe was thrust through the serosa and muscularis, whereupon the required amount of inoculum was introduced into the submucosa. The accuracy of introducing the inoculum into the submucosa by such a blind method may be questioned, but the difference in the sense of resistance offered to the penetrating needle by the serosa and muscularis, on the one hand, and the submucosa, on the other, is so great that one is never in doubt regarding the position of the needle point. Moreover, it is almost impossible to enter the pyloric lumen with the needle for the reason that the mucosa bulges in advance of the needle point until it contacts the opposite wall, giving rise to the danger of penetrating the posterior wall rather than to that of entering the lumen. Owing to one accidental inoculation of the abdominal incision, special precaution against such contamination was thereafter strictly observed.

Altogether, 51 dogs and 8 young cats were inoculated. Only animals which looked healthy and well nourished were selected, in order that the results might be based on trustworthy material. The age of the first 4 cats ranged from 3 to 9 months, while that of the last 4 ranged between 7 weeks and 3 months. The first 14 dogs in the series were kept in individual cages. The other dogs were assigned to two fairly large rooms joined by a passage; here the dogs adapted themselves to confinement, played together and were maintained under a strict quarantine. The cats were kept in individual cages in a sunny room with good ventilation.

A milk diet was given during the first three to five days following inoculation and thereafter a general diet consisting of 1 part of fresh ground beef heart and 2 parts of a prepared food in a dry form, composed of cereal, bone, meat and minerals. It was found desirable to kill the animal to be examined in the morning when the stomach was empty. Immediately after death the cardiac and pyloric ends of the stomach were ligated, and the lumen was filled by injection of a 3.5 per cent solution of formaldehyde. The stomach was then removed and placed in a jar containing a solution of formaldehyde of the same strength. A careful autopsy was made on every animal in the series, either when it was killed or as soon as possible after death. Four hours later the stomach was opened along either the lesser or the greater curvature and carefully examined, after which the extent and intensity of the macroscopic lesion were recorded. Later the specimens were cut into small blocks of tissue, embedded in paraffin, sectioned and stained with hematoxylin and eosin; over 30 blocks of tissue were studied in serial section.¹⁶

OBSERVATIONS

The inoculation of human strains of *E. histolytica* into the gastric submucosa gave rise to the formation of gross gastric lesions in 36 dogs and 5 cats (69.49 per cent of the series); in the remaining 15 dogs and 3 cats (30.51 per cent) a lesion could not be demonstrated even with the

16. This work was carried out in the technic room of the department of anatomy under the direction of Dr. O. F. Kampmeier.

aid of a hand lens. The lesions were without exception confined to the stomach; the small and large intestine and the liver failed to show any gross evidence of extension. During the course of the experiment 21 dogs and 2 cats died; 6 dogs of this group presented, in addition to the gastric lesion, some intercurrent disease, while the remainder contained only the amebic lesion. At varying intervals after inoculation 30 dogs and 6 cats were killed. The duration of observation varied from one to one hundred and three days, the average being slightly over four days for the animals that died and about thirty-five days for those that were killed. The majority of the animals displayed only a moderate constitutional reaction to the inoculation; their appetite returned on the first or second day following the injection, and diarrhea or loss in weight was usually not observed with the infection. There were a number of animals that died while the amebic process was still in an early stage, 10 within twenty-hours and 8 within four days, and these showed a strong general reaction to the inoculation.

The earliest lesions observed in the series occurred in dogs that died within twenty-four hours. A lesion of such brief duration consisted of a region of hemorrhagic exudation in the submucosa with moderate congestion of the overlying mucosa; the involved layers presented considerable induration but no apparent thickening or elevation. Surrounding the area of exudation in the submucosa was a zone of infiltration consisting of lymphocytes, plasma cells, polymorphonuclear leukocytes and histiocytes. The gastric pits and glands were filled with a cellular debris, and many of the chief cells were detached from the basement membrane. There was marked engorgement of the involved capillaries and vessels. One dog of this group presented an hourglass constriction of the stomach at the level of the lesion, set up by tonic contraction of a segment of circular muscle fibers, and the hemorrhagic exudation in the lesion extended into the submucosa overlying the constriction.

Circumscribed nodules appeared at primary sites in dogs that died within three days after inoculation, and the various forms are displayed in figure 1. The extravasation of serum and red blood corpuscles into the interstices of the submucosa was sufficiently extensive to increase the depth of this layer to two or three times its normal thickness, and peripheral to it was a region of infiltration with numerous polyblasts, plasma cells and polymorphonuclear leukocytes. Many sections contained large depositions of hematin from the hemoglobin set free by disintegration of the red corpuscles. In some lesions the hemorrhagic exudation extended into the connective tissue septums of the inner part of the muscular coat, causing swelling of the septums and converting the adjoining smooth muscle tissue into a homogeneous hyaline material. The mucosa at the apex of the nodule was transformed into a mass

of granular detritus, while at the periphery the outline of the deeper part of the glands was still visible. The muscularis mucosae was surprisingly well preserved in sections of many of the lesions. The vessels were congested or thrombosed. An occasional displaced parietal

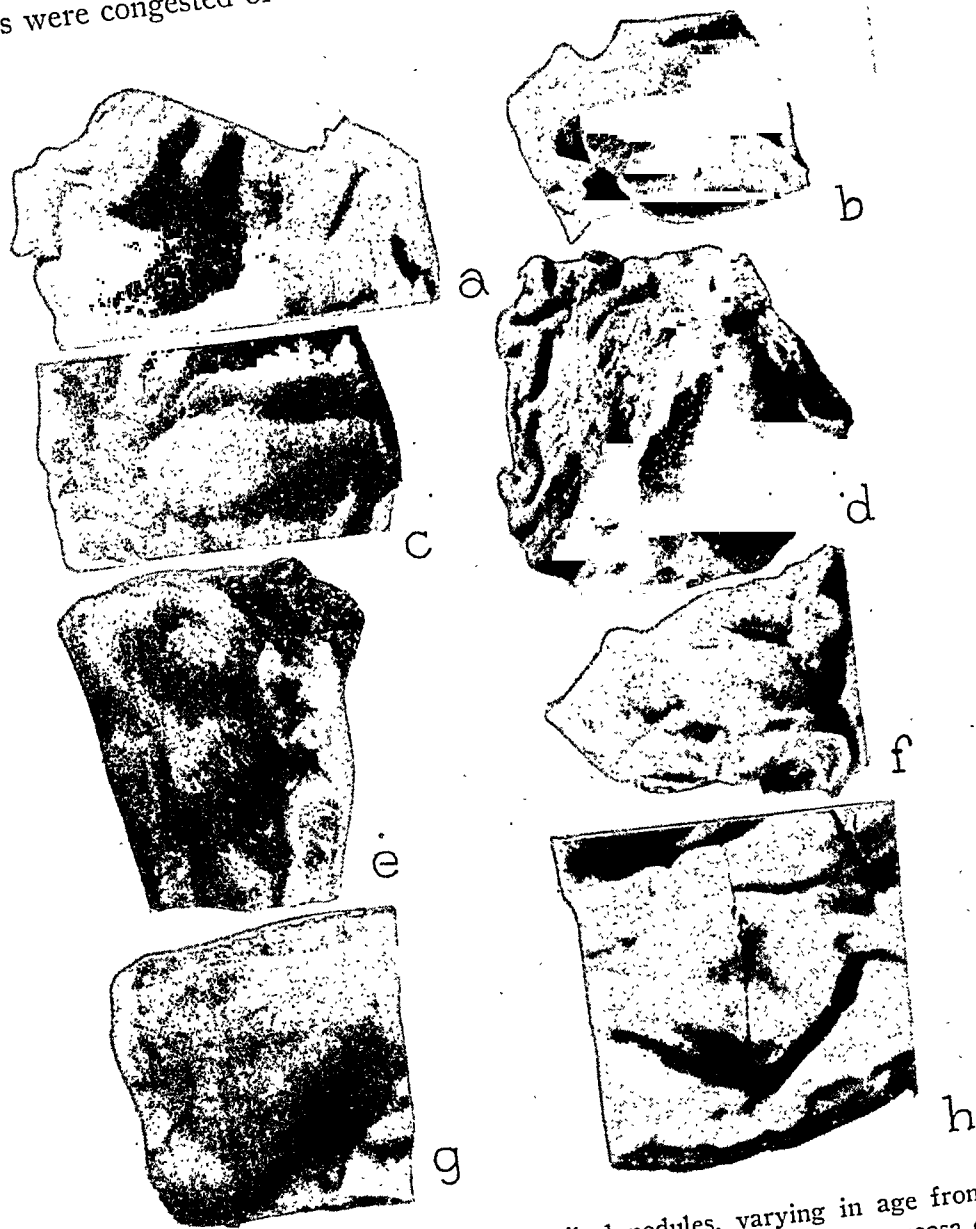


Fig. 1.—A group of large circumscribed nodules, varying in age from forty-eight to seventy-two hours, occurring at sites of inoculation. The mucosa covering these specimens was deeply congested, with early softening. (f) The mucosa was in part disintegrated. (h) The submucosa was converted into a soft, moist mass of gray necrotic material, which gave rise to the umbilication in the central part of the intact mucosa.

cell of a fundus gland was found to bear some resemblance to an ameba, especially when surrounded by a clear space, and then caution was necessary to make the proper differentiation.

The soft nodular lesion portrayed in figure 1 *h* occurred in a dog that lived only three days; the involved submucosa was reduced to a moist, slightly coherent mass, which was perhaps responsible for the umbilication marking the center of the intact mucosa. The liquefied mass in section showed a dense cellular infiltrate in its central part and a fibrinous exudate at the periphery, in which the inflammatory cells were less dense; the muscularis mucosae remained unbroken.

Nodules arose also at secondary sites, and most of these developed as an extension of the process in older specimens. They occurred as single and multiple lesions with considerable variation in size and shape, as shown in figure 2. The specimens exhibited in *a* and *b* came from dogs killed eighty-nine and one hundred and three days after inoculation, in which the pyloric mucosa, even after the most careful inspection, failed to reveal a primary lesion; the mucosa covering each lesion presented an apical ulceration, while the serosa contained an eccentric sloughing area of pinhead size. The multiple lesions in *c* and *d* came from a dog that died within thirty-six hours after injection; there were thirty or more small nodules distributed in a definitely linear manner over the entire gastric mucosa on, as well as between, the folds.

The overlying mucosa in most of these secondary nodules was hyperemic, and at the apex of the lesion it contained a minute ulcerated area of pinhead size or larger, from which a sinuous tract led downward to a cavity of flasklike profile, filled with a hemorrhagic exudate or a brown gelatinous débris. The cavity was usually located in the deeper part of the mucosa, but in several instances it occupied the submucosa and the sinus leading to it and then perforated the muscularis mucosae. A few parietal cells were seen in the débris either in the tract or in the cavity, the staining qualities of which were well preserved. Except for the region embracing its proximal end, the sinuous tract was surrounded by tissue in which there was little, if any, cellular inflammatory reaction. In one lesion there occurred a small, irregularly shaped cavity in the mucosa which failed to present a communication with either the surface or a contiguous cavity.

Ulceration became manifest as early as the third day after inoculation, and the destructive phases of this process continued to be predominant in some of the primary lesions for as long as eighteen days. The ulcers in figure 3 indicate the various stages of development in these lesions of primary origin. In many of them the cavity communicated with the surface through a wide opening, but this was not true of all the lesions. Each of the three ulcers in line in the specimen shown in *c* presented a slitlike opening on the surface, which was continuous with a piriform excavation below. This specimen came from a dog killed on the ninth day on account of a protrusion of the greater omentum through the

lower half of the abdominal wound; the most distal of the three ulcers extended to within 0.5 cm. of the pyloric sphincter.

The crater of the primary ulcer usually extended down through the mucosa and submucosa; in the more susceptible animals it involved a

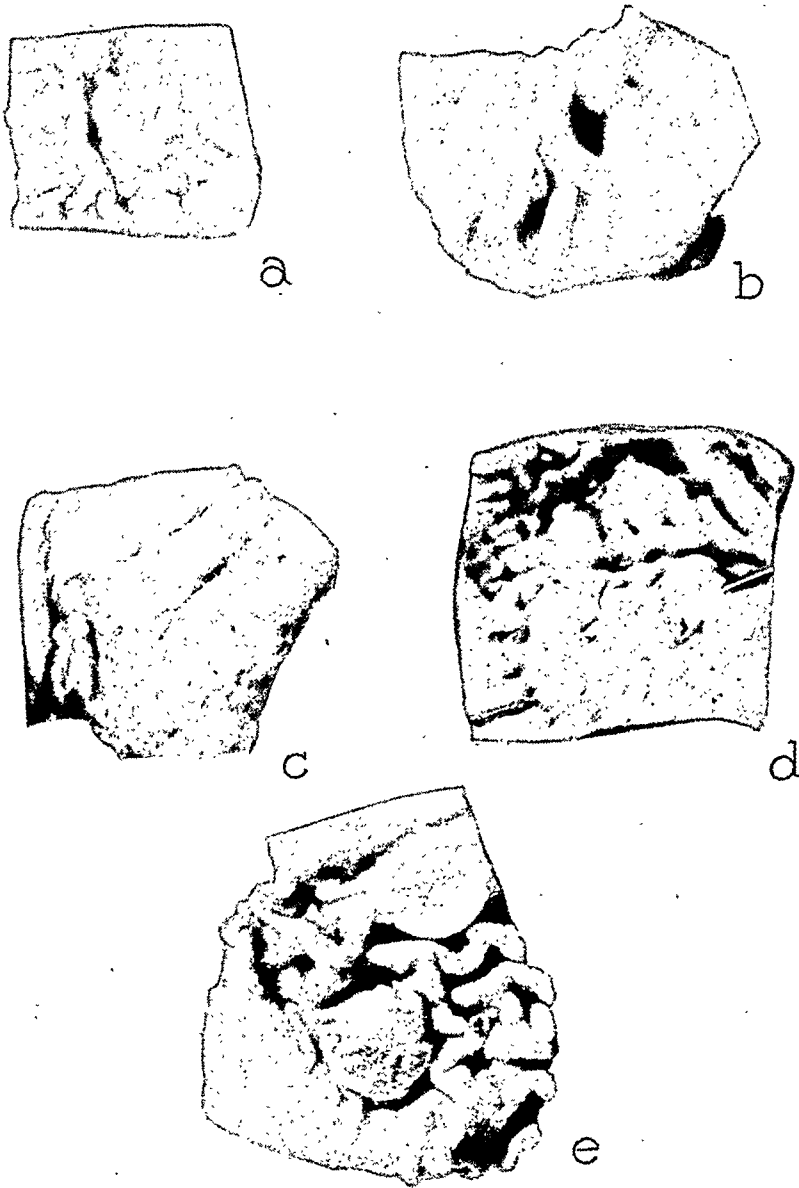


Fig. 2.—A group of nodular lesions occurring at secondary sites, many of them presenting minute apical ulcerations: (*a* and *b*) specimens taken from dogs eighty-nine and one hundred and three days after inoculation; (*c* and *d*) multiple superficial lesions in a dog which died within twenty-four hours after inoculation; (*c*) a lesion located within 5 cm. of the cardiac sphincter and associated with many small superficial erosions as shown in figure 4 *d*.

large part of the muscularis. An extensive gangrenous process took place in a lesion of eighteen days (*c* in fig. 4) in which the floor was composed only of thickened serosa. In the early lesions the crater was

filled with necrotic material, only partially separated from the base and sides. The spread of the amebic process was rapid and extensive in the submucous layer, where the advancing infiltration and softening of the tissue led to undermining of the mucosa above. The mucosa in the central

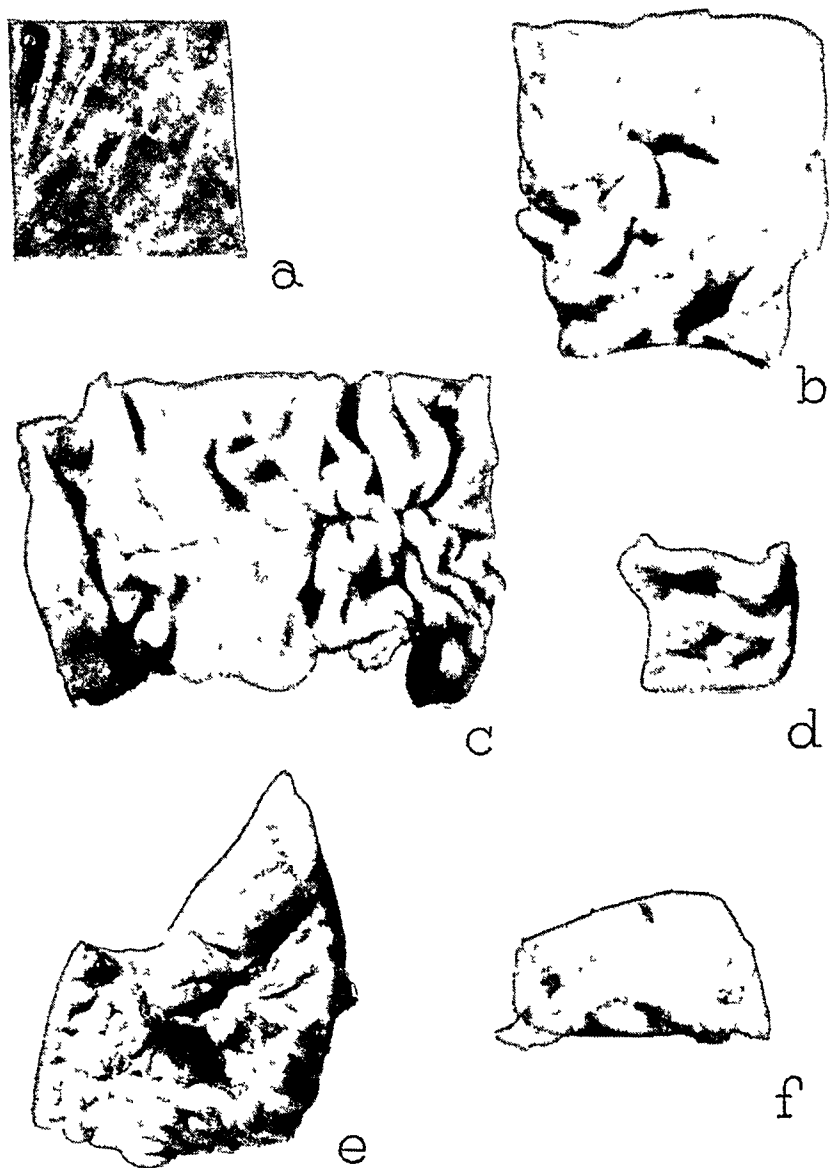


Fig. 3—A group of ulcers appearing at primary sites. (a) lesion with punched-out appearance; (b) induration and undermining of margins; (c) three button-hole ulcers in line in a dog killed after nine days; (e and f) excavations filled with grayish brown necrotic material, which was firmly adherent to the floor and sides.

portion was cut off from its nutrition and cast off as a slough, and with further destruction in the submucosa the undermined mucosa at the edge of the ulcer became everted. Two inflammatory zones could sometimes

be distinguished in the transition from normal tissue to the completely necrotic area. The proximal zone was marked by a serous exudate with numerous red blood corpuscles and the peripheral zone by an extensive infiltration of polyblasts, plasma cells and polymorphonuclear leukocytes.

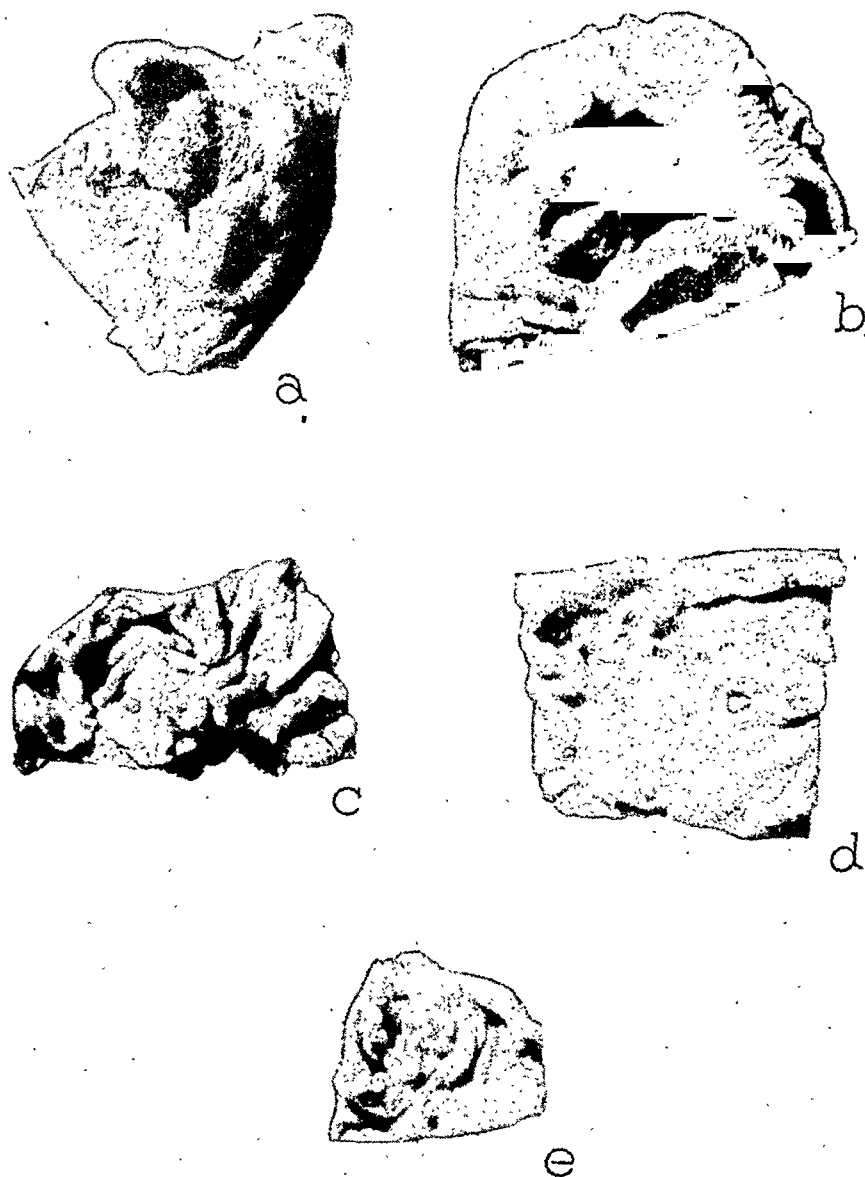


Fig. 4.—Larger gangrenous ulcers are illustrated in *a* to *c*; the lesion in *c* is eighteen days old and the thin, almost translucent floor is composed of only a thickened serosa; *d* and *e* show multiple superficial erosions.

of which cells the last predominated when cocci and bacilli were present in the lesion. The submucosa was greatly thickened in the inflammatory area, which caused an elevation of the edge of the ulcer. Large ulcerative processes were observed in 8 dogs and 4 cats, in which the necrotic

material was cast off, with the formation of an excavation of variable size and shape, and the floor was covered usually with an adherent grayish brown material; sections often revealed two layers in the floor, a superficial stratum of fibrin with a rich cellular infiltration and a deeper layer of numerous fibroblasts and newly formed capillaries. The vessels in the wall of the ulcer were congested or thrombosed and occasionally presented obliterating endarteritis; the walls of vessels were usually edematous and infiltrated with inflammatory cells, and the resistance which they displayed to the lytic action of *E. histolytica* was at times striking.

Amebas were found in sections of only two specimens in the series. The first was a large gangrenous ulcer appearing at the site of inoculation in a cat which died three days after injection and in which the postmortem examination was delayed about twelve hours. The amebas, which were lodged in the wall of the lesion, contained granular cytoplasm, surrounded by the characteristic clear space, and a nucleus which was spherical and somewhat inconspicuous (fig. 5 *A-C*). The second specimen was a primary ulcer occurring in a dog killed four days after inoculation, in which the tissues were fixed immediately after death (fig. 6 *A* and *B*). The amebas were present in the fibrin of the floor of the ulcer; the nuclear structure, owing perhaps to the increased intensity of the stain, was clearly visible, particularly the layer of fine chromatin granules lining the achromatic nuclear membrane internally and the karyosome with the clear zone surrounding it. Secondary infection occurred in only three ulcerated lesions, in which there was great destruction of tissue and in which the fixation was delayed from eight to twelve hours; the floor and sides of these three ulcers were uneven and ragged, containing numerous cocci and bacilli, with a predominance of polymorphonuclear leukocytes.

Ulcerated lesions, like the nodular lesions, occurred also at secondary sites. The age of these lesions was determinable only from the stage of their development. Multiple superficial erosions were observed in two specimens (fig. 4 *d* and *e*), one from a dog and the other from a cat, killed, respectively, after twenty-one and thirty-five days. The erosions in the dog occurred without a demonstrable primary lesion, while those in the cat were associated with a large primary sloughing ulcer. The lesions were distributed over the whole stomach, and in the specimen from the dog there was also a large nodule with partial mucosal disintegration close to the cardiac sphincter; an occasional buttonhole ulcer occurred among the erosions in both specimens. Each erosion consisted of an area of disintegration involving the gastric glands, around which

was usually a dense cellular infiltration. The secondary lesions associated with the older lesions are described in connection with healed lesions.

The destructive and inflammatory processes in ulceration were gradually replaced by regeneration in the various tissues, and phases

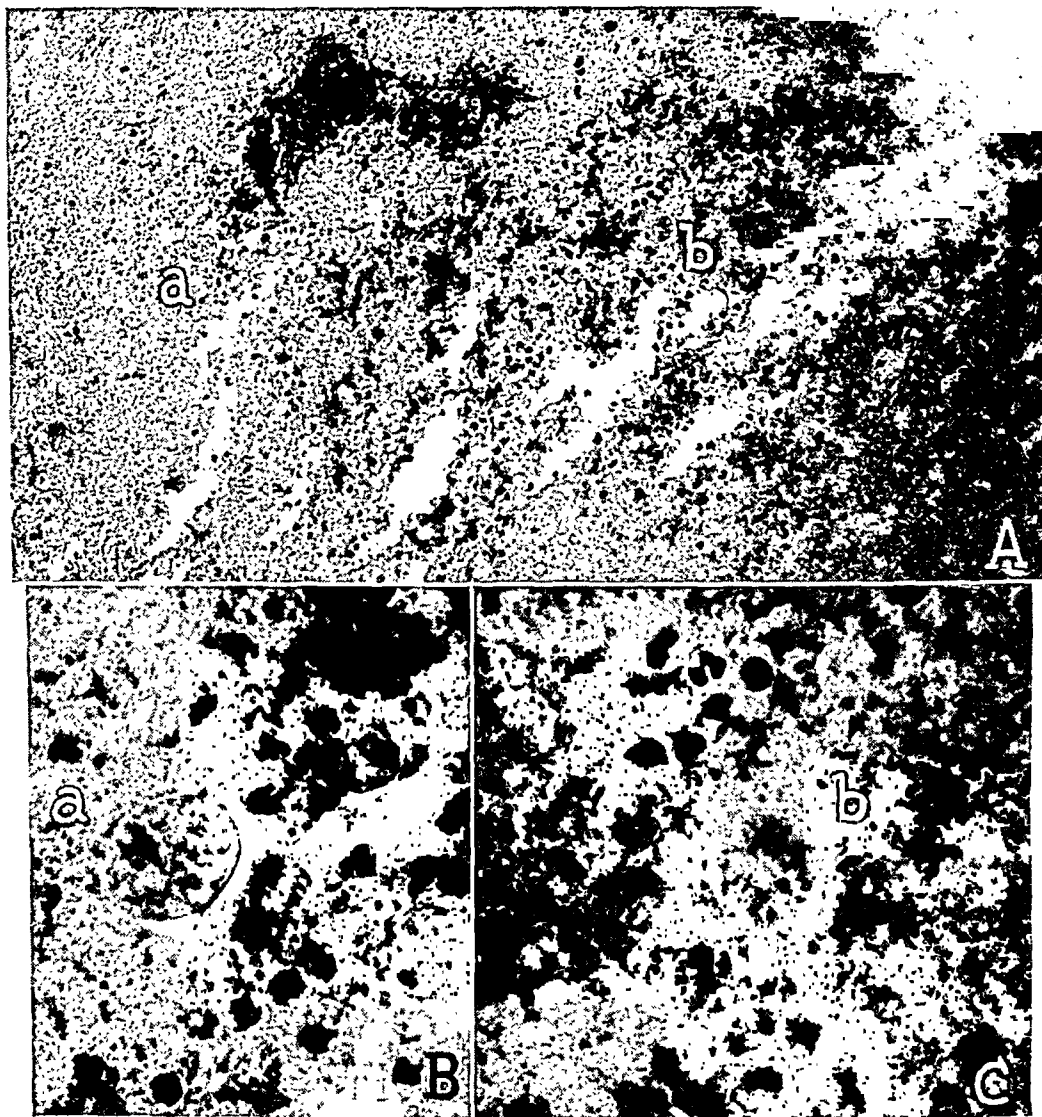


Fig. 5.—*A*, section ($\times 90$) of the side of the ulcer displayed in figure 3*f*, in which the submucosa contains two amebas (*a* and *b*); *B* and *C*, the same section showing the amebas under higher power ($\times 800$).

of all these processes were occasionally seen in one and the same specimen. The epithelium regenerating from the surface and from the gastric glands appeared as early as the fourth day as flattened cells lining the cavity of the ulcer, and at about the same time the first signs of organization became evident in the fibrin of the floor. The earliest

lesion to give the gross appearance of being completely healed occurred in a dog killed after fourteen days (fig. 7 *a*), but on histologic study a small part of the floor was found to be still uncovered with epithelium.



Fig. 6.—*A*, section ($\times 91$) of the ulcer shown in figure 3 *a*, in which several amebae are embedded in the fibrinous floor; *B*, the same section under higher power ($\times 700$), exhibiting at *a* the nuclear structure in one of the amebae; the chromatic granules lining the achromatic nuclear membrane are visible; the two amebae, *b* and *c*, to the left and below are out of focus and do not show their nuclei distinctly.

Healed lesions were found in 8 dogs and 1 cat of the series, the duration ranging from sixteen to ninety-eight days (fig. 7 *a-h*).

The epithelial hyperplasia presented an interesting aspect. As the new epithelium grew out over the granulation tissue in the floor of the

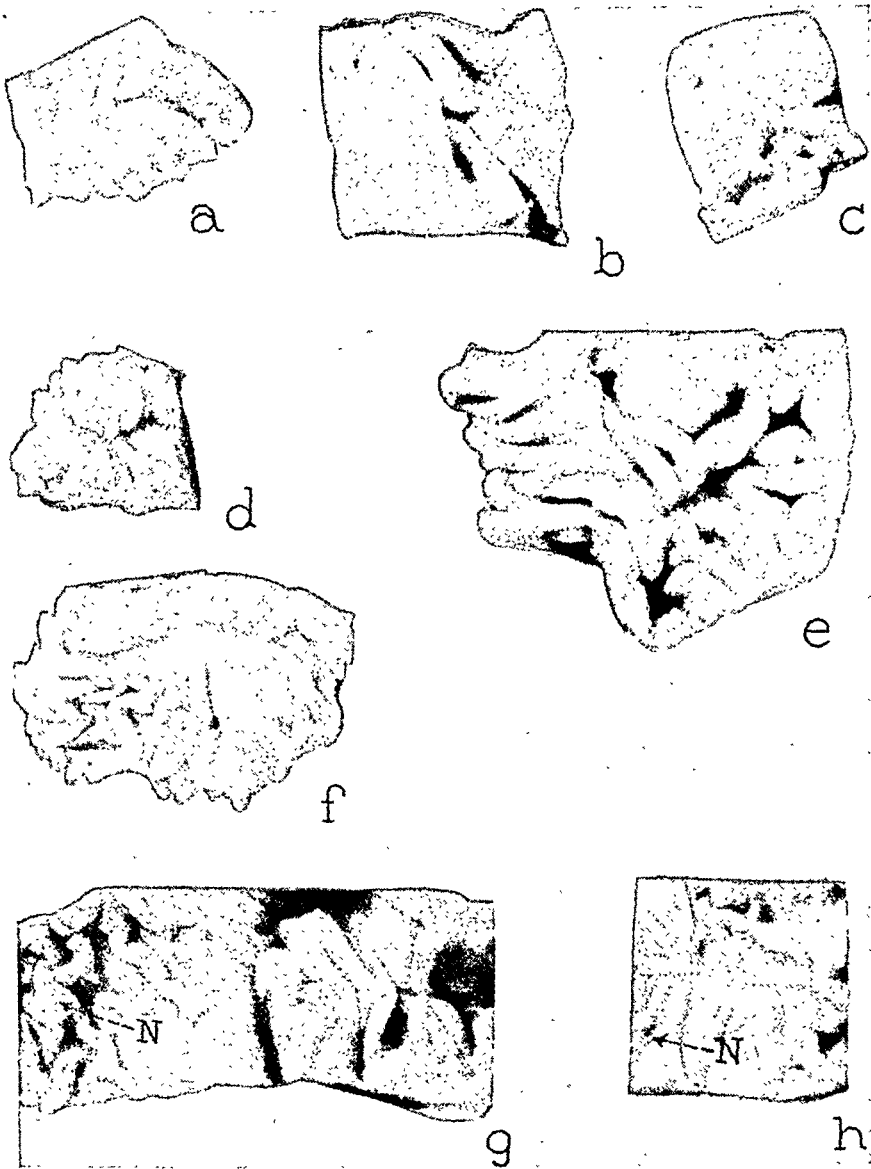


Fig. 7.—A group of healed primary lesions from sixteen to ninety-eight days old; the scars in *g* and *h* are associated with active secondary nodules at *N*, the latter containing a minute apical ulceration.

ulcer, the latter became heaped up by the advancing epithelial edge from each side into an irregular projecting mass, which was reduced only after it became covered with the newly formed epithelium. The cells in the single layer of epithelium in the floor gradually changed from an atypical flattened to a columnar shape; from this layer invaginations

developed later in a downward direction, giving rise to shallow pits and glands with chief cells. The organization in the fibrin of the floor took place in the presence of numerous infiltrating lymphoid cells, histiocytes and leukocytes. Isolated strains of muscle fibers grew up into the organizing tissue of the floor, forming a scant network of muscle tissue at this level.

As healing approached completion, the gastric pits became deeper and the glands more convoluted, and below the bases of the glands there appeared occasionally a newly formed lymphoid follicle; up to the end of the period of observation the glands remained less tortuous than the normal, and the number of glands per unit area was less than that of the normal pyloric mucosa. No regeneration of the muscularis mucosae was observed in any of the specimens. The base of the healed ulcer was usually pigmented and depressed, and the edges were puckered, elevated and everted; the eversion in some lesions was so marked that the edges approximated one another and hid the floor from view. The edge of the old ulcer was always identified histologically by following the muscularis mucosae to its eversion and fixation in the new fibrous tissue.

The completion of the healing process in the primary lesion was not found to signify the end of the amebic process in the wall of the stomach. Three healed lesions were each accompanied by a recent secondary lesion; two of these are displayed in figure 7 *g* and *h*. The first, twenty-three days old, presented two linear scars separated by a normal fold of mucosa, and about 4 cm. proximal to the scars was an active nodular thickening, covered with congested mucosa. The second specimen, sixty-three days old, contained a well healed irregular scar, and less than 1 cm. from its proximal end occurred a nodule with a minute apical ulceration, which led down through a sinus to a hemorrhagic region in the submucosa. In the third specimen (fig. 2 *a*), which was eighty-nine days old, there was no evidence of a primary lesion, but on the anterior surface of the corpus, close to the antrum and the greater curvature, the mucosa presented an active buttonhole ulcer with a hemorrhagic area in the submucosa.

COMMENT

The inoculations led to the production of a large number of gross lesions as seen at autopsy. A wide variety of lesions was obtained in which to study the evolution of the amebic process in the stomach. Many phases of development from the acute to the completely healed stage were seen, and in the active lesions occurred numerous forms of transition from erosions of the most superficial kind to excavations of a deep and extensive nature. The earliest reaction to the inoculation consisted of a hemorrhagic exudation into the submucosa, which became so extensive that the involved layer, as well as the mucosa above, was deprived of

its blood supply. The necrosis which followed in the devitalized mucosa was in part due to the action of the gastric juice; this action was later responsible for the conversion of the hemoglobin into acid hematin, the formation of which caused the black discoloration in the tissues of the floor and sides of the excavation. The infectious process set up by the inoculations in the more susceptible animals became widespread, manifesting extensive lateral involvement and spreading to all layers of the gastric wall. The destructive process differed, however, from the processes produced by other types of experimental infection of the stomach, particularly in the dog, in that it failed to heal promptly; in some primary lesions it remained active for as long as eighteen days, and if the lesions appearing at secondary sites are included in the consideration, the duration of activity exceeded three months. Signs of healing were apparent, of course, as early as the fourth day in the less susceptible animals, and the earliest completely healed lesion was obtained on the sixteenth day after inoculation.

The histologic study of the lesions revealed the unexpected fact that the extent of necrosis in the stomach was out of all proportion to the number of amebas found in the tissues. Most of the sections failed to show amebas. In the sections from only two lesions were amebas identified; the one specimen occurred in a dog and the other in a cat, and about 10 to 15 amebas were found in the serial sections of each. No cause can be cited for the disappearance of the amebas from the gastric lesions. Hara¹⁷ made a study of the disappearance of amebas within the intestines of cats and found that the amebas were greatly affected within an hour after death of the infected animal and that after two hours the great majority of them were almost ready to disappear. Hiyeda and Suzuki¹⁸ observed that the time of disappearance was longer than that quoted by Hara and concluded that the amebas disintegrate in the tissues as a result of postmortem degeneration.

The possibility exists that the gastric juice exercises a destructive action on the amebas after death of the host. The specimens containing amebas in our series were fixed at different intervals, the one from the dog immediately after death and the one from the cat about twelve hours after death. In the sections from the specimen fixed immediately after death the surface epithelium between the gastric pits was in an excellent state of preservation, and the same might have been expected of the amebas unless amebas are more sensitive to the action of gastric juice.

From the experimental results it follows that *E. histolytica* was able to survive in its vegetative form in the base and sides of each of two

17. Hara, S., cited by Hiyeda and Suzuki.¹⁸

18. Hiyeda, K., and Suzuki, M.: *Am. J. Hyg.* **15**:809, 1932.

lesions, unaffected by the gastric juice during the life of the host. This characteristic of the amebas in resisting the action of hydrochloric acid seems almost incontestible, because amebas with typical structural arrangement in the nucleus and cytoplasm were recovered three and four days after inoculation. The experiment by Dobell, which demonstrated the resistance of the trophozoites of *E. histolytica* to acid in vitro, finds corroboration in our experiments in vivo. Critical data with which to explain the phenomenon of resistance to acid in *E. histolytica* were not obtainable. It is possible that the cultured forms colonized and reproduced in the looser, more vascular tissue of the submucosa and by an adaptation to the antagonistic conditions of the stomach acquired an immunity which enabled them to continue the invasive process even in the presence of gastric juice. Different types of trophozoites are recognized by Hegner, Johnson and Stabler,¹⁹ but Arnold²⁰ stated that he knew of no instance in which parasites underwent changes in virulency due to nutritional influences and emphasized that all investigations point toward changes in the natural defensive powers of the host.

The part which the amebas took in the production of lesions in our experiments should be considered. The association of amebas with only two of the lesions in the series may not be sufficient proof that the relation between *E. histolytica* and the primary lesions is one of cause and effect. Further evidence, however, of this causative relation is found in the close resemblance which all the primary lesions displayed, whether with or without amebas, to the typical amebic lesion of the intestine. The role played by the secondary invaders in the development of amebic lesions is perhaps not as great in the stomach as it appears to be in the intestine. The deterring action of gastric juice against all forms of bacteria is well known, and it is a significant fact that the cocci and bacilli found in the sections of our series occurred only in specimens in which fixation was delayed three hours or longer after death.

The development of lesions at secondary foci, with all the characteristics of amebic lesions, constituted one of the most interesting phenomena of the experiment. Early secondary lesions occurred in close proximity to the primary site as long as three months after the initial inoculation. Though the ultimate course of the primary lesion was unquestionably toward cicatrization, with final restoration of the mucous membrane, it is apparent that the infectious process set up by the inoculations did not become extinct within a period of three months; the presence, moreover, of a healed lesion did not indicate a restitutio ad

19. Hegner, R. W.; Johnson, C. M., and Stabler, R. M.: *Am. J. Hyg.* **15**:394, 1932.

20. Arnold, L.: *Am. J. Digest. Dis. & Nutrition* **1**:351, 1934.

integrum, because of the occurrence of secondary lesions. At this juncture it may be of interest to consider the pathway pursued by the infectious process in its distribution from primary to secondary sites. Most of the secondary lesions were either nodular formations situated close to the surface in the mucosa or excavations communicating with the surface by means of sinuous tracts. These structural features indicate that the spread of the infectious agent was along the surface. One specimen, however, contained a small cavity in the mucosa which communicated neither with the surface nor with any adjacent lesion. But this apparently buried cavity is no evidence against surface dissemination, because it is quite possible that the infectious agent might perforate the surface or the wall of a gastric gland without leaving the slightest trace of its path.

No tunnel-like communications were seen between the multiple lesions at secondary sites, nor did these lesions display any tendency toward coalescence. The absence of any inflammatory reaction in the tissues surrounding the sinuous tract or the excavation may be significant in explaining the mechanism of extension of the lesion; indeed, the failure in the formation of a fibrinous exudate or in cellular infiltration may predispose to a perforation of the gastric wall.

That any essential dependence may exist between *E. histolytica* and the lesions formed at secondary sites may be questioned on the ground that none of the sections of these lesions contained amebas. In this connection it should be emphasized that the spontaneous occurrence of acute or chronic ulceration in healthy dogs and cats, if it appears at all, is rare. Turck²¹ reported that in necropsies on 189 healthy and 82 diseased dogs peptic ulceration was not found once. Mann²² did not find a single lesion of the gastric mucosa in more than 200 practically normal dogs and cats examined post mortem. Ivy²³ with 900 healthy dogs found an acute gastric ulcer in a single dog (0.09 per cent), while petechial hemorrhages and superficial hemorrhagic erosions occurred, respectively, in 4.1 and 2.68 per cent of his series. The great morphologic similarity between the basic lesion of amebic disease and the secondary lesions in our series supports the theory of amebic origin of the latter. The activity of the amebas during the period between inoculation and the occurrence of the secondary lesions becomes a matter of interest if an amebic origin is assumed. In this interval it is quite possible that an equilibrium may exist between the vitality of the amebas and the defense of the host, and the appearance of a secondary lesion may indicate a breaking down of this equilibrium and the beginning of the invasive process.

21. Turck, F. B.: *J. A. M. A.* **46**:1853, 1906.

22. Mann, F. C.: *J. Exper. Med.* **23**:203, 1916.

23. Ivy, A. C.: *Arch. Int. Med.* **25**:6, 1920.

A considerable number of animals failed to show any gross lesion at autopsy. The failure in the development of a reaction after inoculation may be due to changes in either the amebas or the host. The microscopic study of the inoculum preceding inoculation did not reveal any morphologic changes in the cultured forms. There was variation in the motility of the amebas, but no relationship could be established between this variation and the intensity of the reaction in the host. The virulency of the amebas failed to show any change during the five months of cultivation; in fact, the largest and most extensive ulcer occurred in a dog inoculated at the very end of the series.

The resistance which the tissues of the host possess toward the toxic action and the penetrative ability of *E. histolytica* must be recognized. Unfortunately, little is known concerning the nature of the reaction in the unsusceptible animal. Kessel²⁴ expressed the belief that the resistance of the host is a more important factor than the difference in the racial virulence of the parasite, and Craig²⁵ held that a natural immunity exists in some persons. There is a likelihood that the local immunity against the histolytic process is sufficiently effective to allow the formation of only very minute lesions, which heal so rapidly and promptly that no gross evidence of a tissue reaction is discernible. The procedure of inoculating 2 and sometimes 3 animals in succession on the same day from the same culture tube afforded an opportunity of studying the variation in resistance of hosts while the virulence of the parasites remained relatively constant. Three cats of approximately the same age and weight were inoculated in this manner and killed nine, sixteen and thirty-five days after inoculation; a buttonhole ulcer occurred at the site of inoculation in the first, no visible lesion in the second and a number of superficial erosions in the third.

The fact that the stomach of the dog and of the cat has been demonstrated to be experimentally infectible with *E. histolytica* renders it less certain that the human stomach always escapes invasion on exposure to amebic infection. It is claimed that *E. histolytica* is the only tissue-invading ameba which has been found in the digestive tract of man. Arnold,²⁶ studying the bacterial flora of the gastrointestinal tract, observed that under certain conditions the stomach loses the power of controlling the bacterial life within its lumen. He illustrated the difference in the nature of the bacterial flora of the stomach in the normal condition, in the hypofunctioning state and in the acute gastric upset, and suggested that a similar mechanism may underlie the control of

24. Kessel, J. F.: J. A. M. A. **90**:1089, 1928.

25. Craig, C. F.: Amebiasis and Amebic Dysentery, Springfield, Ill., Charles C. Thomas, Publisher, 1934.

26. Arnold, L.: Am. J. M. Sc. **186**:471, 1933.

parasitic infection. In our series the amebic lesion resembled in many respects the gastric ulcer occurring in man. Some of the manifestations common to both are: the localization of the lesion to the stomach; the predominance of single over multiple lesions, with the absence of any coalescence of the latter; the tendency of the process to extend through all the layers of the gastric wall, with the danger of perforation, and, finally, the marked tendency toward recurrence. These characteristics suggest a relation between *E. histolytica* and the genesis of gastric ulcer in man.

SUMMARY

In 51 dogs and 8 cats the pyloric submucosa was inoculated with three strains of cultured forms of *E. histolytica* isolated from man. The inoculations gave rise to gross lesions in 36 dogs and 5 cats. Death occurred in 15 dogs and 2 cats in which no lesions were found other than the experimental gastric lesions. Sections of two specimens in the series showed amebas with a typical structural arrangement of nucleus and cytoplasm, indicating that *E. histolytica* can exist in its vegetative form in the base and sides of a lesion in the gastric wall, unaffected by the action of gastric juice during the life of the host. Early secondary lesions occurred in close proximity to the primary lesions as long as three months after inoculation.

The many characteristics which the resulting lesions possessed in common with the so-called peptic ulcer of the human stomach suggest the possibility of a causative relation between gastric ulcer in man and *E. histolytica*.

INTESTINAL LESIONS IN CONGENITAL SYPHILIS

A HISTOLOGIC STUDY, WITH A REPORT OF THREE ADDITIONAL
CASES, IN ALL OF WHICH SPIROCHETES
WERE IDENTIFIED

RIGNEY D'AUNOY, M.D.

AND

BJARNE PEARSON, M.D.

NEW ORLEANS

Involvement of the intestine in congenital syphilis is distinctly unusual. Even in 1900, when the incidence of frank syphilitic lesions was considerably more frequent than it is at the present time, Oberndorfer¹ was able to collect from the literature only 24 cases, to which he added 1 instance of his own. Fraenkel² found only 3 cases in 19,000 autopsies. Among 8,856 consecutive postmortem records from the Charity Hospital of Louisiana at New Orleans reports of only 3 cases were found. These constituted 1.3 per cent of the 230 cases of syphilis in infants who came to autopsy in that institution during the period from Jan. 1, 1929, to Jan. 1, 1937.

The frequency with which gastrointestinal syphilis is found in congenitally syphilitic infants varies with different authors and obviously depends on the amount of material available and the care with which each body is examined. Thus Foerster³ found the typical lesion only once in 36 cases of congenital syphilis, whereas Mracek⁴ found it ten times in 200 cases, and Chiari⁵ noted it seven times in 111 cases. The statistics of Birch-Hirschfeld,⁶ collected in 1875, show an incidence of 12.5 per cent, and Oluf Thomsen's⁷ statistics, collected in 1928, show

From the Department of Pathology and Bacteriology of the School of Medicine of Louisiana State University and from the Charity Hospital of Louisiana at New Orleans.

1. Oberndorfer, S.: *Virchows Arch. f. path. Anat.* **159**:179, 1900.
2. Fraenkel, cited by Wile, D.: *Arch. Dermat. & Syph.* **3**:372, 1921.
3. Foerster, A.: *Würzburg. med. Ztschr.* **4**:1, 1863.
4. Mracek, F.: *Vrtljschr. f. Dermat.* **10**:209, 1883.
5. Chiari, cited by Schneider, P.: *Verhandl. d. deutsch. path. Gesellsch.* **23**:177, 1928.
6. Birch-Hirschfeld, F. W.: *Arch. d. Heilk.* **16**:166, 1875.
7. Thomsen, O.: *Pathologisch-anatomische Veränderungen über die congenitale Syphilis bei dem Foetus und dem neugeborenen Kind*, Copenhagen, Levin & Munksgaard, 1928.

an incidence of 8.3 per cent. The incidence of 1.3 per cent (3 of 230 cases) which we have found is low, but this is not surprising. Intestinal syphilis is practically always associated with more severe visceral lesions, and the frequency of such lesions, according to syphilographers, has been markedly decreased during recent years by the rather universal application of intensive antisyphilitic therapy.

The purpose of this paper is twofold: to put on record the 3 cases of intestinal syphilis observed, in all of which spirochetes were identified, and to describe the gross and microscopic lesions. Our justification for making the report lies in the fact that to date the number of cases on record in which spirochetes have been identified is very small, and in the fact that none of these cases is recorded in either the English or the American literature. Indeed, so far as we have been able to determine, only a single case of intestinal syphilis is on record in this literature. The report of this case, which was made by Yampolsky and Fowler⁸ in 1936, concerns a newborn, full term girl who died an hour after birth. Blood from the umbilical cord gave a strongly positive Wassermann reaction, and a roentgenogram of the bones was suggestive of syphilis. Ascites was present, and slight adhesions between two loops of jejunum were noted. The syphilitic lesions were in the upper portion of the jejunum; they did not involve the mucosa and, according to the authors, resembled tubercles. Apparently spirochetes were not searched for. The case was somewhat unusual because of the character of the gross lesion and the absence of other visceral lesions.

In some 50 per cent of the reported cases of intestinal syphilis, the process was observed in stillborn or macerated fetuses. In 27 per cent the children were born alive but death occurred shortly after birth or within twenty-four hours. A few others lived from ten days to three or four weeks. Ljunggren's⁹ subject lived to the age of 2 years, and Roth's and Birch-Hirschfeld's each lived to the age of 3 years.

In recent years, since the specific etiologic agent of syphilis has been identified, special stains for spirochetes have been used in the study of the tissues of the syphilitic infants in some reported cases of intestinal syphilis, but the organism has not always been identified. Ku¹⁰ found spirochetes in only 2 of the 4 cases which he reported, though in all 4 cases the lesion itself, as well as the associated visceral involvement, was characteristic. Spirochetes were first identified in the intestine by Versé¹¹ in 1906. Since then they have been found by Fraenkel,¹²

8. Yampolsky, J., and Fowler, C. D.: *J. M. A. Georgia* **25**:154, 1936.

9. Ljunggren, A.: *Arch. f. Dermat. u. Syph.* **2**:141, 317 and 547, 1870.

10. Ku, D. Y.: *Virchows Arch. f. path. Anat.* **280**:852, 1931.

11. Versé, M.: *Med. Klin.* **2**:626, 653 and 682, 1906.

12. Fraenkel, E.: *München. med. Wchnschr.* **54**:156, 1907.

Thomsen,¹³ Warstat,¹⁴ Ku¹⁰ (in the 2 cases just referred to) and Kernau.¹⁵ To these cases we add 3 cases, in each of which spirochetes were found in abundance in the intestinal lesions, as well as in the other organs.

In nearly all cases of congenital intestinal syphilis there is associated involvement of one or more other organs. In more than half of the reported cases the liver was involved, and the lungs were involved in slightly less than half. Weber's osteochondritis was noted in half the cases and cutaneous involvement in nearly a third, syphilitic pemphigus being the most prominent lesion. In Roth's patient, the 3 year old child just referred to, the intestinal lesion was associated with encephalitis and periostitis. Periostitis usually occurs considerably later in life, and encephalitis is also uncommon. Another case of interest was reported by Foerster;³ in this case there was an associated fibrous inflammation of Glisson's capsule, an association, according to Thomsen, which occurs in 11.61 per cent of all cases of congenital syphilis.

Of particular interest is the association of intestinal syphilis and syphilitic lesions of the stomach. In 8 of the 25 cases collected by Oberndorfer¹ this association was noted, and it was also present in 6 of the 15 cases of gastric syphilis, congenital in 7 of them, which he put on record in the same communication. Oberndorfer was impressed with the frequency of the association, as was Brunner,¹⁶ though the latter considered that intestinal syphilis is more frequent than gastric syphilis.

By far the most common lesion in congenital intestinal syphilis is a raised yellow plaquelike band. These bands occur at irregular intervals on the intestinal wall and tend to encircle the bowel completely. They stand out prominently if the bowel is at all distended when the abdomen is opened. In these areas, in over half of the reported cases multiple ulcerations were observed, and perforation was frequent. The latter was noted in Mracek's,⁴ Jürgens'¹⁷ and Ku's¹⁰ cases, as well as in a case of our own, and in all it was followed by generalized peritonitis. Even when perforation has not occurred, adhesion of adjacent coils of bowel may occur at points of involvement by a plastic exudate, and peritonitis can be present without perforation. This happened in 2 of our cases, as well as in cases reported by Ku¹⁰ and Fraenkel.¹²

13. Thomsen, O., cited by Schneider, P.: *Verhandl. d. deutsch. path. Gesellsch.* **23**:177, 1928.

14. Warstat, G.: *Virchows Arch. f. path. Anat.* **212**:195, 1913.

15. Kernau, T.: *Centralbl. f. allg. Path. u. path. Anat.* **64**:5, 1935.

16. Brunner, C.: *Tuberkulose, Aktinomykose, Syphilis des Magen-Darmkanals*, Stuttgart, Ferdinand Enke, 1907; in Billroth, T., and Lücke, A.: *Deutsche Chirurgie*, *ibid.*, 1907, no. 46e, p. 333.

17. Jürgens, cited by Ku.¹⁰

Other types of lesion have also been described. Birch-Hirschfeld reported diffuse thickening of the intestinal wall. Peyer's patches may be so extensively involved that they are visible on gross inspection, and lesions resembling small gummas may also be identified.

Schneider¹⁸ expressed the belief that intestinal syphilis occurs most frequently in the upper portion of the small intestine, and Herxheimer,¹⁹ that the jejunum is the most frequent site. The recorded cases seem to show a slight predilection for the lower part of the ileum and for the ileocecal region. In about a third of the cases the lesions are chiefly in the ileocecal region; in the remaining cases the distribution is about equal in the upper part of the ileum and the lower and upper parts of the jejunum. Localization in the duodenum is not common and when it occurs is most marked about the papilla of Vater. Ku¹⁰ described 2 cases in which both the jejunum and the duodenum were involved. Roth (quoted by Oberndorfer¹) reported another unusual case in which the process was localized in the transverse colon. In our cases the lesions occurred variously in the lower part of the jejunum and in the ileum, in the upper part of the jejunum and lower part of the ileum, and throughout the small bowel.

From the histologic standpoint syphilitic lesions in the intestinal tract have frequently been described as gummas. In 1913 Aschoff pointed out that true granuloma is rare, and advanced the view that the specific miliary foci in congenital syphilis are chiefly the result of an acute inflammatory reaction such as occurs in abscess formation. Schneider¹⁸ divided the specific foci in congenital syphilis into three categories: miliary necrosis, abscess-like miliary foci and true miliary granuloma.

The histologic lesions consist essentially of necrosis of the mucosa and submucosa plus severe endarteritis. Proliferation of young connective tissue is usually abundant and involves the whole intestinal wall, especially when it is in the diffuse sclerotic form. Abscess-like miliary foci may also be seen, as in all our cases and in those reported by Ku.¹⁰ Herxheimer²⁰ considered this lesion to be due to an especially heavy infection with spirochetes. Granulation tissue is stimulated, and leukocytes wander in from the periphery. In some areas the mucosa may be intact but may show severe atrophy and replacement by granulation tissue.

Spirochetes, when they are found, are most abundant at the margins of the areas of necrosis. They are also abundant in the vascular walls and in the perivascular area. In Ku's¹⁰ cases they were most frequent in the mucosa and in the perivascular tissue.

18. Schneider, P.: *Verhandl. d. deutsch. path. Gesellsch.* **23**:177, 1928.

19. Herxheimer, G.: *Ergebn. d. allg. Path. u. path. Anat.* **12**:499, 1908.

20. Herxheimer, G.: *Verhandl. d. deutsch. path. Gesellsch.* **23**:144, 1928.

The 3 cases of congenital intestinal syphilis observed at the Charity Hospital during the eight year period ending in 1937 are presented herewith. Only observations relevant to congenital syphilis in general and intestinal syphilis in particular are recorded.

REPORT OF CASES

CASE 1.—The body was that of a premature, stillborn Negro boy, well developed but poorly nourished, measuring 40 cm. in length and weighing $3\frac{1}{2}$ pounds (1,533 Gm.). The skin was slightly macerated. The lungs were firm in consistency, leathery to the touch and grayish pink; they presented no aeration. The liver was markedly enlarged, and its capsule was smooth and glistening. The tibia showed syphilitic osteochondritis.

In the wall of the small intestine were numerous small cheesy purulent areas. The loops of the bowel were adherent to one another, and many of the ulcerated areas had perforated, so that intrainstestinal communications were numerous.



1. Photograph of the small intestine in a case of congenital intestinal syphilis. Note the tendency of the plaquelike lesions to encircle the bowel.

The gross diagnosis was congenital syphilis with involvement of the intestine, liver, spleen, lungs and bones. Spirochetes were found in abundance in all the internal organs.

CASE 2.—The body was that of a premature Negro girl who lived for twenty-seven minutes. It was well developed and well nourished. The crown-rump length was 25 cm., and the crown-heel length, 37 cm. It weighed $4\frac{1}{2}$ pounds (2,041 Gm.). There was a slight icteric tinge over the whole body.

The lungs were pale pink, with areas of consolidation. The liver, which weighed 125 Gm., was firm and light brown. The surfaces exposed by cutting were light brown and mottled, and the markings were indistinct. The spleen, which weighed 40 Gm., was dark red. The surfaces exposed by cutting were firm and fibrous. The pancreas was yellow, slightly enlarged, nodular and firm. The surfaces exposed by cutting revealed distinct lobulations. Section through the femur showed distinct syphilitic osteochondritis.

The upper coils of the small intestine were markedly distended, and there were fine adhesions between the cecum, the lower part of the ileum and the parietal peritoneum, which was thickened. Yellow plaquelike elevations, about 0.5 cm. in width, encircled the ileum at intervals of about 4 cm. throughout its length. At least half of the jejunum was similarly involved. There were occasional areas of necrosis, and corresponding areas of the mucosa showed definite ulcerations. The cecal mucosa was thickened. No ulcerations were present in the stomach, duodenum or esophagus.

The gross diagnosis was congenital syphilis with involvement of the intestine, liver, pancreas and bone. All the internal organs showed spirochetes in abundance.

CASE 3.—The body was that of a premature Negro boy who lived for three hours. The crown-rump length was 28 cm., and the crown-heel length, 43 cm. The weight was 4 pounds (1,814 Gm.). A slight icteric tinge was present, but there were no gross lesions of the skin. The abdomen was distended, especially in the upper portion.

The left lung weighed 30 Gm., and the right, 35 Gm. Both were light pink and rather firm, with whitish areas throughout. Little air-containing tissue could be seen. The cut surface felt rather firm.

The liver extended to the left, and almost to the anterior superior spine of the ileum. It weighed 165 Gm. and was dark brownish and rather firm. The surfaces exposed by cutting were dark brown, firm and homogeneous, and normal markings were absent. The pancreas, which was approximately normal in size and weight, was slightly nodular and very firm. The surfaces exposed by cutting were also firm and slightly nodular. The bone showed typical syphilitic epiphysitis.

Neither the esophagus nor the stomach revealed any evidence of ulceration or of tumor. The duodenum also was normal except for marked accentuation and outpouching of the papilla of Vater. There were numerous elevated circular thickenings of the upper portion of the jejunum and the ileum, which were approximately from 0.5 to 1.5 cm. in width and from 0.5 to 0.75 cm. in thickness. In some areas these bands encircled the bowel; in others they were most prominent on the antimesenteric border. On the jejunum these lesions were about 1.5 cm. apart. They became less numerous as the ileum was approached and in its first portion were about 10 cm. apart. In the distal part of the ileum they were more numerous and were 2 or 3 cm. apart. Some of these areas exhibited central ulceration, but the majority showed only a thickened plaquelike elevation. The appendix, cecum, transverse colon, sigmoid and rectum revealed no abnormalities.

The gross diagnosis was congenital syphilis with involvement of the lung (pneumonia alba), liver, intestine, pancreas and bone. Spirochetes were found in abundance in all the internal organs.

MICROSCOPIC OBSERVATIONS IN CASES 1, 2 AND 3

Since the histologic changes in all 3 cases were practically the same, they will be described jointly, with particular reference to those in the intestine. The other organs revealed the histologic appearances specific for the diagnosis of syphilis. Present in all 3 cases was marked interstitial pancreatic fibrosis—a change mentioned in not more than 15 per cent of the other reported cases. To judge from our observations, this is the most common, definite and reliable pathologic change in congenital syphilis. We have found it in all cases in which there was visceral involvement, and Thomsen¹³ stated that it occurred in 86.9 per cent of all cases of congenital syphilis.

The liver, spleen and lungs showed syphilitic involvement in all 3 cases, the lungs exhibiting pneumonia alba in all. All these organs showed spirochetes in abundance.

Sections through the involved areas of the intestinal wall showed absence of mucosa in the ulcerated areas. Both the mucosa and the submucosa showed necrosis of the fibroblastic tissue, only a few well stained plasma cells and endothelial leukocytes being seen. At the periphery this necrotic area merged into an area

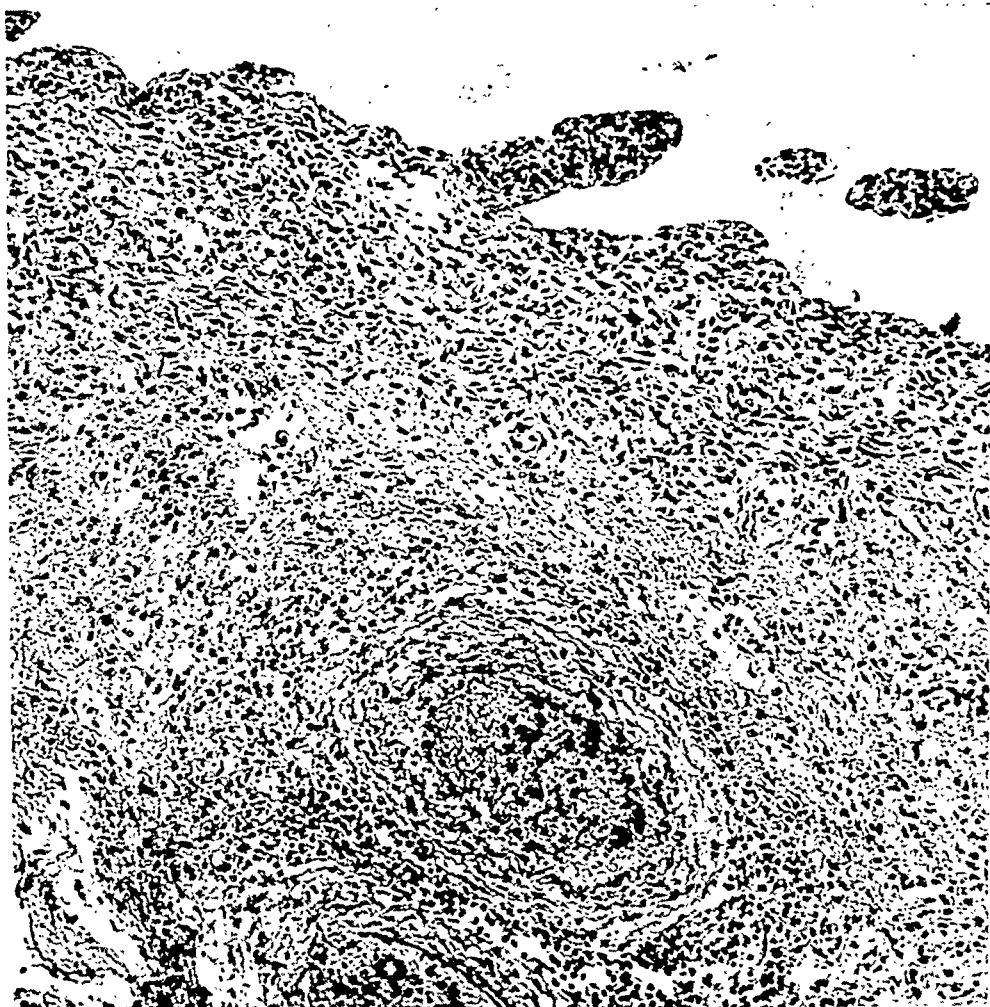


2. Bowel wall showing infiltration of the mucosa and submucosa with plasma cells and fibroblastic tissue. Miliary abscess-like foci, such as can be seen in the lower portion, are an important early finding in intestinal syphilis.

composed chiefly of young fibroblasts and many newly formed capillaries. Within the meshes of this newly formed granulation tissue were many plasma cells and endothelial leukocytes. In some miliary areas of necrosis the granulation tissue was very faint. Some cellular infiltration with leukocytes could be seen. In the area of granulation tissue pyknosis of the nuclei of cells was frequent. The muscular portions, as well as the adventitia, showed many dilated vessels, which were filled with red cells. Some of the red cells had extravasated into the surrounding

tissue and were there broken up into hemosiderin. The hemosiderin, together with the many fat cells present throughout, was probably responsible for the yellowish color. The muscular layers of the intestine were well infiltrated with leukocytes, which showed pyknosis. Plasma cells were present in abundance. In some places the muscular layers had been replaced by young granulation tissue. Endarteritis was marked.

The earlier lesions in intestinal syphilis could be studied best in areas of transition from normal mucosa to more involved portions. Here the plasma cells

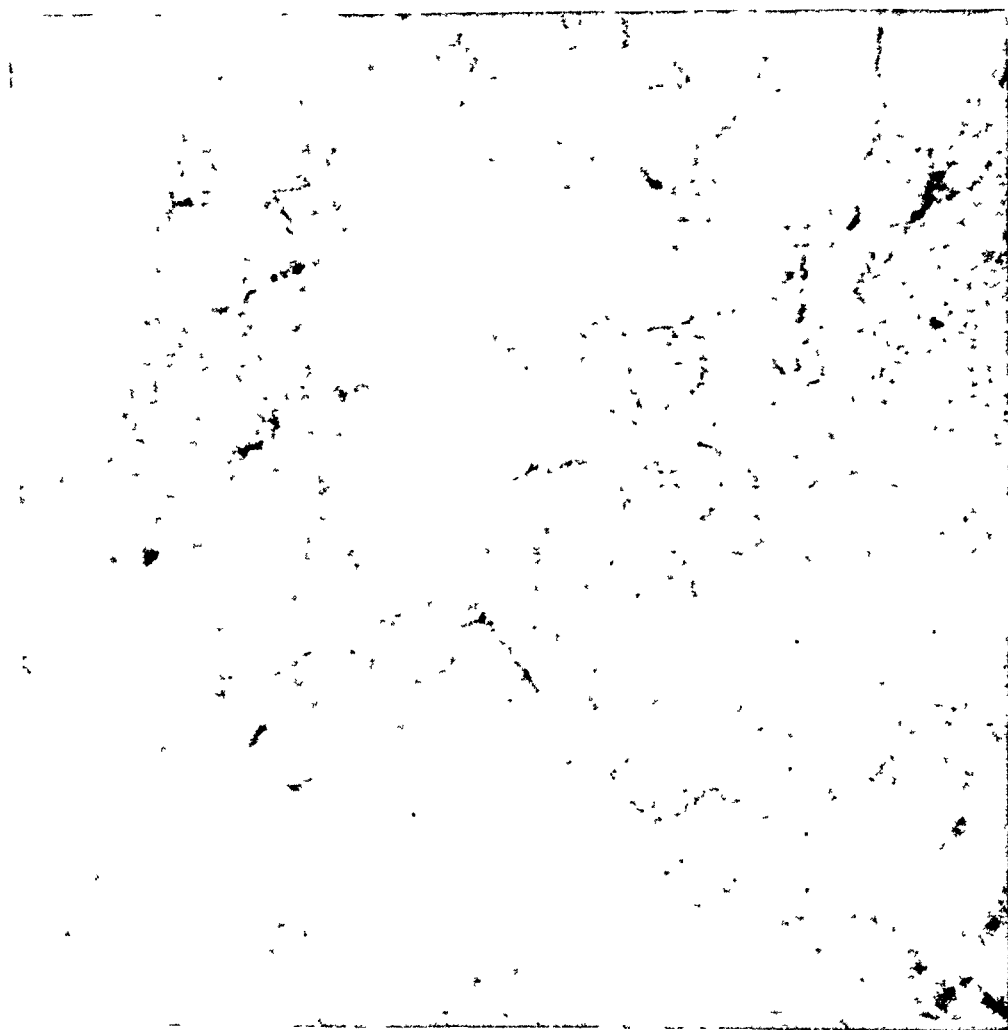


3. Older lesion showing presence of more mature fibroblasts and replacement of the intestinal layers with definite fibrous tissue. Note the beginning fading-out and necrosis of the fibroblasts. Note also the thickening of the wall and the splitting of its layers in the blood vessel.

were numerous, and while the deeper mucosal glands could still be seen they tended to disappear as plasma cells became more numerous and as an early fibroblastic reaction occurred. The mucosa then became transformed into young granulation tissue.

In the submucosa could be seen many miliary syphilomas, corresponding to the kind described by Schneider as "abscess-like" miliary foci. We believe that such lesions represent early stages of the process and that many later become necrotic. Various

combinations of the necrotic and the abscess-like forms were seen. The abscess-like areas were present only when granulation tissue was present, and no giant cells could be seen in any portion. The vessels in the submucosa and in the more normal portions showed extreme dilatation. Little perivascular infiltration could be seen. The newly formed fibroblastic tissue tended to encircle the small vessels in such a manner that on first glance it seemed to be an actual part of the wall of the vessel. The arrangement, however, was apparent and not real; usually there was a small space between this tissue and the arterial wall proper.



4. Section from the wall of the ileum, stained by Levaditi's method, showing numerous spirochetes, some undergoing degeneration (oil immersion).

Spirochetes in abundance were easily demonstrated by silver stains throughout the bowel wall. Degenerated club forms could also be seen. The spirochetes in sections were most numerous around the small vessels and within the walls of vessels. They were present throughout the various tunics of the bowel, even in the necrotic areas, where, however, they were less numerous. They lay in the same planes as the muscle fibers, and the contrast between the longitudinal and the circular distribution was striking.

SUMMARY

Congenital syphilis with intestinal involvement was uncommon even before the era of general specific therapy and is even less frequent at this time. More than 75 per cent of all cases are observed in macerated stillborn infants and infants who live less than twenty-four hours. Some type of visceral involvement is always associated with the intestinal lesion; most usually it is an involvement of the liver or of the lungs. Involvement of bone is also frequently associated with the intestinal lesion.

The most common and most characteristic intestinal lesion is a raised yellow plaquelike band which encircles the bowel. Generalized peritonitis may follow ulceration and perforation of these areas but may also occur in absence of frank rupture. The syphilitic process is chiefly confined to the small intestine; it has a special predilection, from the standpoint of number of lesions and intensity, for the last portion of the ileum.

The lesions include necrosis of the mucosa and submucosa of the involved areas, miliary syphiloma and abscess-like foci. Vascular dilatation, followed later by fibroblastic reaction, probably occurs earliest in the mucosa and submucosa. Subsequent necrosis is frequent. Replacement of the whole bowel wall with fibroblastic tissue is common in the more severely involved portions.

Spirochetes, when they can be identified, are seen in all the layers of the bowel and are especially prominent in the perivascular tissues and in the vessel wall. In the muscular coats they tend to lie in the direction of the muscle fibers.

We have put on record, with a complete histologic study, 3 cases of congenital intestinal syphilis, in all of which spirochetes could be demonstrated.

PATHOLOGIC CHANGES IN THE NERVOUS SYSTEM IN YELLOW FEVER

LEWIS D. STEVENSON, M.D.

NEW YORK

In a study of small pieces of brain tissue from 14 persons who died of yellow fever, it was found that there was marked perivascular hemorrhage in various parts of the brain in most of these persons. In view of this finding it was thought desirable to obtain, if possible, the whole brain in cases of this disease in order to determine the localization of the hemorrhages and to study any other change from the normal that might be present in the brain in yellow fever. Accordingly the whole brain was obtained and studied in 20 cases.

METHODS

Sections were made as far as possible through the superior frontal gyrus, the paracentral lobule, various parts of the temporal lobe, the occipital lobe, the cerebellum, the periventricular and subthalamic region at the level of the mamillary bodies, the midbrain, the pons and the medulla, and through the spinal cord in 3 cases. In some cases sections were made also through the lenticular nucleus and the insula. Some of the brains were partly spoiled for study because of postmortem softening, so that the regions studied were not identical in all cases.

Masson's trichome stain was used to demonstrate hemorrhages, and all embedded sections were embedded in pyroxylin. Hematoxylin and eosin stains were also used to demonstrate cellular exudate in the meninges or in the brain. Preparations of myelin sheaths were made in many cases by Loyez's¹ method. Weigert's stain for elastic tissue, with counterstaining by Van Gieson's method, was used in a study of the blood vessels in several cases. Nissl's stain was used for a study of the nerve cells in some cases. Frozen sections were stained for neuroglia by Hortega's method as modified by Globus, also for fat and microglia, in all instances.

GENERAL OBSERVATIONS

After fixation the appearance of these brains seldom varied from the normal. In some instances the pathologist who made the necropsy

From the Department of Pathology, Cornell University Medical College, and New York Hospital.

The pathologic tissues examined in this study and the information regarding the illnesses were obtained through the cooperation of the International Health Division of the Rockefeller Foundation. Tissues and data were supplied from Brazil through Dr. Fred L. Soper, Dr. John E. Elmendorf Jr. and Dr. J. A. Kerr, of the staff of the International Health Division, and Dr. Amadeu Fialho, pathologist of the National Department of Health of Brazil; and from Colombia through Dr. J. H. Paul and Dr. E. R. Rickard of the International Health Division.

1. Loyez, M.: *Compt. rend. Soc. de biol.* 62:541, 1910.

stated that the cerebrum and other parts of the brain were congested. Occasionally a few hemorrhagic spots were noted, and in case 6 there was opacity of the cerebrospinal fluid.

Microscopic examination showed fibrous thickening of the arachnoid in several cases, but this was usually normal for the age of the patient. Once or twice slight subarachnoid hemorrhage was encountered.

In all of the cases perivascular hemorrhages were found. Various parts of the temporal lobe and the region at the level of the mamillary bodies were the most frequently affected by the hemorrhages. These hemorrhages were small and usually, although not always, confined to the spaces about small arteries, as well as veins, and often about capillaries. Occasionally a small hemorrhage was noted with no vessel visible from which it was derived. A slight amount of perivascular edema was noted in some of the cases.

There was little evidence of lymphocytic infiltration of the Virchow-Robin spaces or of the meninges. In 9 cases a few infiltrating lymphocytes were seen. No significant loss of myelin was noted in any case. The neuroglia was normal in appearance in all but case 2, and in most of the cases no abnormal amount of fat was seen. The microglia was increased in some cases. The walls of the blood vessels were normal except where hemorrhage had distorted and compressed them. The nerve cells in most of the cases studied varied little from normal, and no inclusion bodies were seen in the cells of the hippocampus or elsewhere.

Much of the hemorrhage in these cases was seen in the sections through the mamillary bodies and optic thalamus. The hemorrhages in this region were usually near the lining of the third ventricle, in the subthalamic region and near the mamillary bodies. Similar hemorrhages were found in this situation in cases of alcoholic encephalopathy, so that this lesion in the subthalamic region is not peculiar to yellow fever. The impression was gained that the lesions in these cases were due to some toxin circulating in the brain and spinal cord in yellow fever. There was little if any evidence of inflammation in the nervous system. There was little suggestion of those changes usually seen in virus infections of the brain.

REPORT OF CASES

CASE 1.—The brain was that of a man aged 62, whose illness lasted seven days and twenty-two hours. At necropsy the meninges were described as slightly congested. There was fibrous arachnoiditis consistent with the age of the patient, with a few red blood cells in the meshes of the pia-arachnoid over the paracentral lobule. The capillaries were congested in this region, but there was no significant edema and no lymphocytic exudate in any of the sections examined. Perivascular hemorrhages were present in the olfactory tract, the paracentral lobule, a site

near the lining of the third ventricle (these were perivenous for the most part), the temporal pole, the pons and the cerebellum. Sections through the cerebellum showed pericapillary and periarterial hemorrhages near the dentate nucleus. The neuroglia, microglia and fat seemed to be normal.

CASE 2.—This brain was from a white man aged 20 years, whose illness lasted five days and twenty-one and a half hours. Hemorrhages were found in the frontal



Fig. 1 (case 2).—Section through the pons showing a slight perivascular exudate of lymphocytes and perivascular edema. Hematoxylin and eosin stain.

and temporal poles. The neuroglia, microglia and fat were normal. In the pons there were a few lymphocytes in the perivascular spaces about one or two vessels (fig. 1). There was some edema in the occipital pole and in the paracentral lobule. This brain was rather badly decomposed in places so that only a limited study was possible.

CASE 3.—The brain was from a white man, aged 55, whose illness lasted six days and five and a half hours. At necropsy the meninges were described as slightly congested. Perivascular hemorrhages were found in the superior frontal gyrus, the hippocampus, the occipital lobe, the insula, the region at the level of the mammillary bodies, the lenticular nucleus, the midbrain, the pons and the cerebellum. There was a slight subarachnoid hemorrhage over the cerebellum. In the midbrain

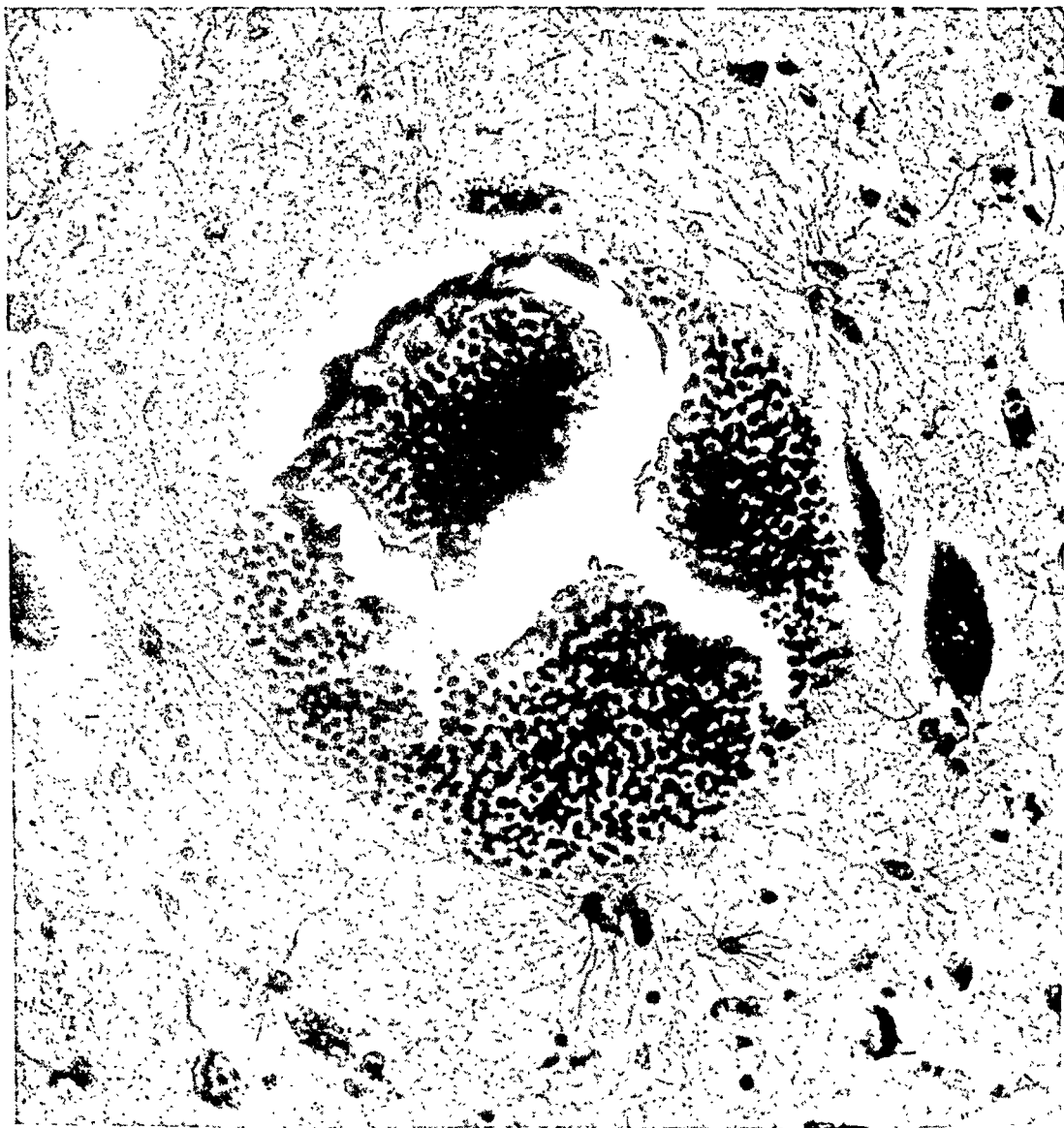


Fig. 2 (case 3).—Frozen section through the pons showing a hemorrhage and at the edge of the hemorrhage some astrocytes in small clusters. Hortega's silver carbonate stain for astrocytes.

a few lymphocytes were present in the adventitia of one or two vessels. Nissl stains of the superior frontal gyrus showed no significant changes. The Weigert stain for elastic tissue with Van Gieson counterstaining showed normal blood vessels. Sections of the superior frontal gyrus stained with Loyez' stain for myelin sheaths showed no loss of myelin. The astrocytes in this brain were increased in number, and figure 2 shows them reacting to the hemorrhage.

CASE 4.—This specimen was from a white man aged 38, whose illness lasted six days and six hours. Perivascular hemorrhages were found in the frontal pole, the superior frontal gyrus, the paracentral lobule, the occipital pole, the region about the third ventricle, the hippocampus, the pons, the medulla and the cerebellum. In the section at the level of the mamillary bodies marked periventricular hemorrhages were present, although none were seen in the mamillary bodies themselves

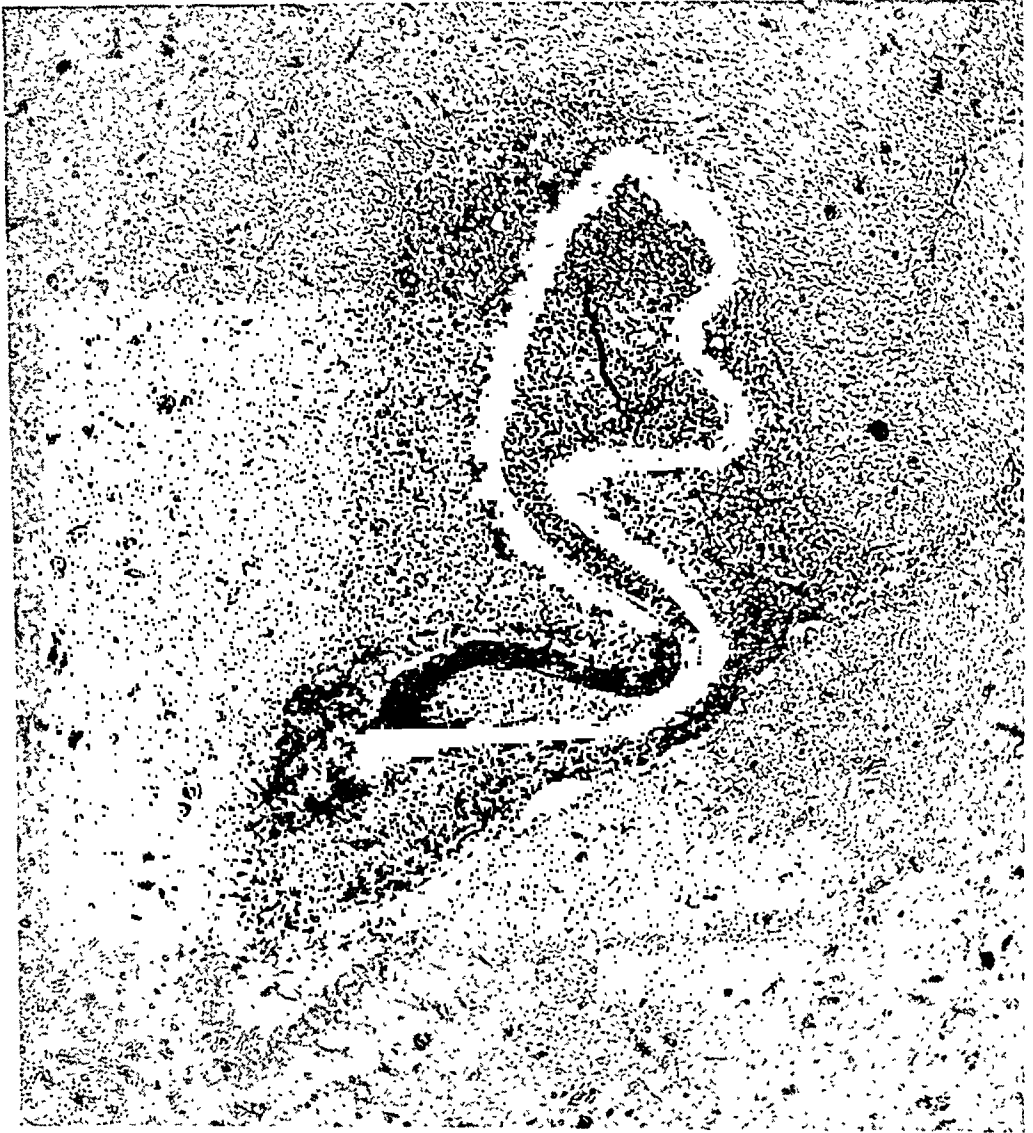


Fig. 3 (case 4).—Section at the level of the mamillary bodies showing a perivascular hemorrhage in the subthalamic region midway between the mamillary body and the massa intermedia. Masson's trichrome stain.

(figs. 3 and 4). There was slight ependymitis in this section, and a few lymphocytes were seen in the adventitia of one or two of the vessels. The astrocytes in the hippocampus and superior frontal gyrus were normal. Nissl stains of the superior frontal gyrus showed no significant change in the nerve cells. Silver carbonate stains of the temporal lobe and superior frontal gyrus showed sclerotic

change in the nerve cells (fig. 5) and an increase in the microglia and fat. Some transitional microglia cells were seen.

CASE 5.—This was the brain of a white man aged 36, whose illness lasted six and a half days. Perivascular hemorrhages were found in the frontal pole, the



Fig. 4 (case 4).—Hemorrhages in the periventricular region at the level of the mamillary bodies. The ependyma lining the third ventricle can be seen. Masson's trichrome stain.

paracentral lobule and the temporal pole. The astrocytes were normal. The microglia and fat were normal.

CASE 6.—The brain was that of a white boy aged 10½ years whose illness lasted six and a half days. Perivascular hemorrhages were found in the frontal pole, the paracentral lobule, the temporal pole, the temporal lobe, the region about

the third ventricle and the medulla. The neuroglia, microglia and fat were normal. There was some fibrous thickening of the arachnoid over the superior frontal gyrus, and perivascular edema was present in this region also.

CASE 7.—The brain was from a white man aged 24, whose illness lasted five days and twenty-one hours. Perivascular hemorrhages were seen in the para-

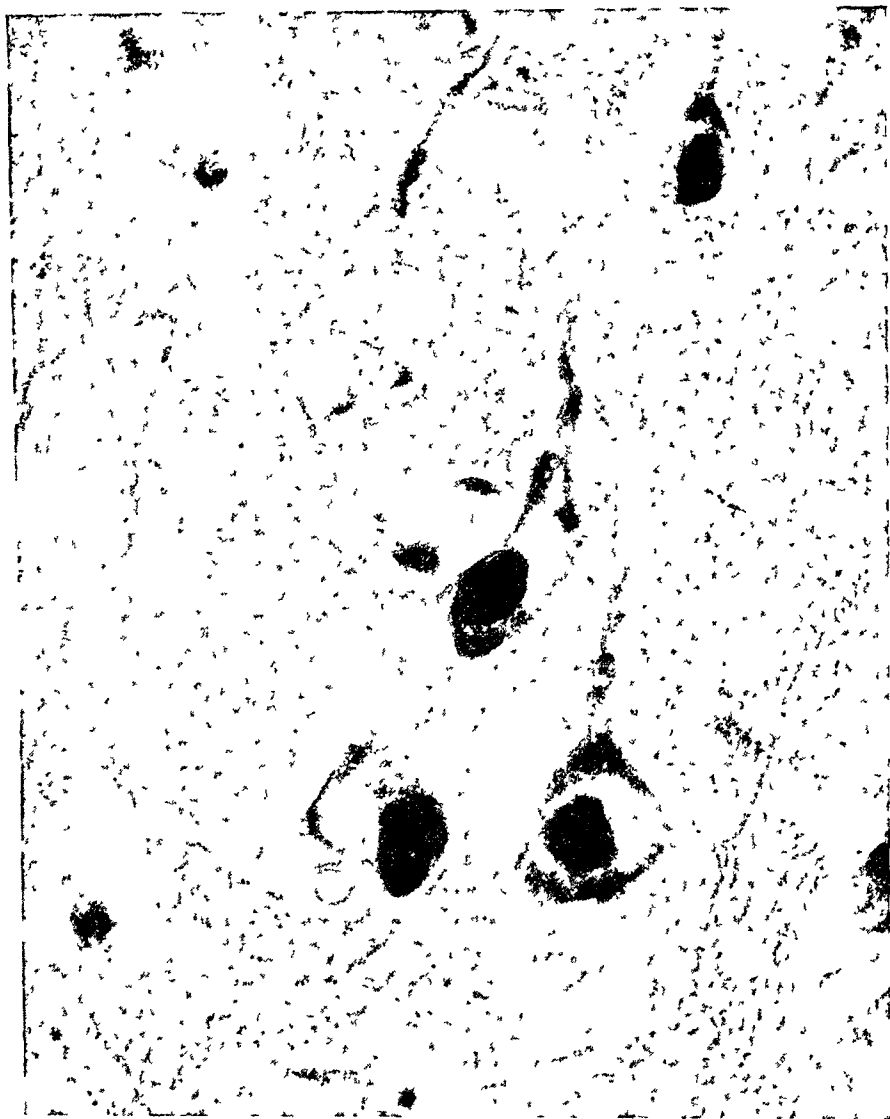


Fig. 5 (case 4) —Frozen section through the superior frontal gyrus showing slight sclerosis of ganglion cells with some increase of fat. Hortega's silver carbonate stain with sudan III.

central lobule, the temporal pole, the region about the third ventricle, the occipital pole and the cerebellum. There was edema in the paracentral lobule. The neuroglia and fat were normal. The microglia was somewhat increased in amount.

CASE 8.—The brain was that of a white man aged 48, whose illness lasted seven days and six hours. At necropsy it was stated that the veins were much dilated

over the cerebrum and that there were small hemorrhagic spots. The cerebellum, pons and medulla were congested. Perivascular hemorrhages were present in the superior frontal gyrus, the paracentral lobule, the temporal lobe, the occipital pole, the tissues about the third ventricle, the midbrain, the medulla, the cerebellum, the



Fig. 6 (case 9).—Section through the hippocampus showing perivascular hemorrhage. Masson's trichrome stain.

olfactory tract and the optic nerve. Weigert's stain for elastic tissue with Van Gieson counterstaining in sections of the superior frontal gyrus showed normal blood vessels. Nissl stains of the superior frontal gyrus showed the nerve cells to be normal. There was some loss of nerve cells in places. Microglia cells were well stained in the temporal lobe and were slightly increased in number; there

were some transitional forms. The fat was increased in amount in the nerve cells of this region and in the perivascular spaces. There was some arteriolar sclerosis in this brain, as well as some fibrous thickening of the arachnoid. There was some subarachnoid hemorrhage over the paracentral lobule, with slight degenerative changes in the nerve cells. The neuroglia in the superior frontal gyrus was normal.

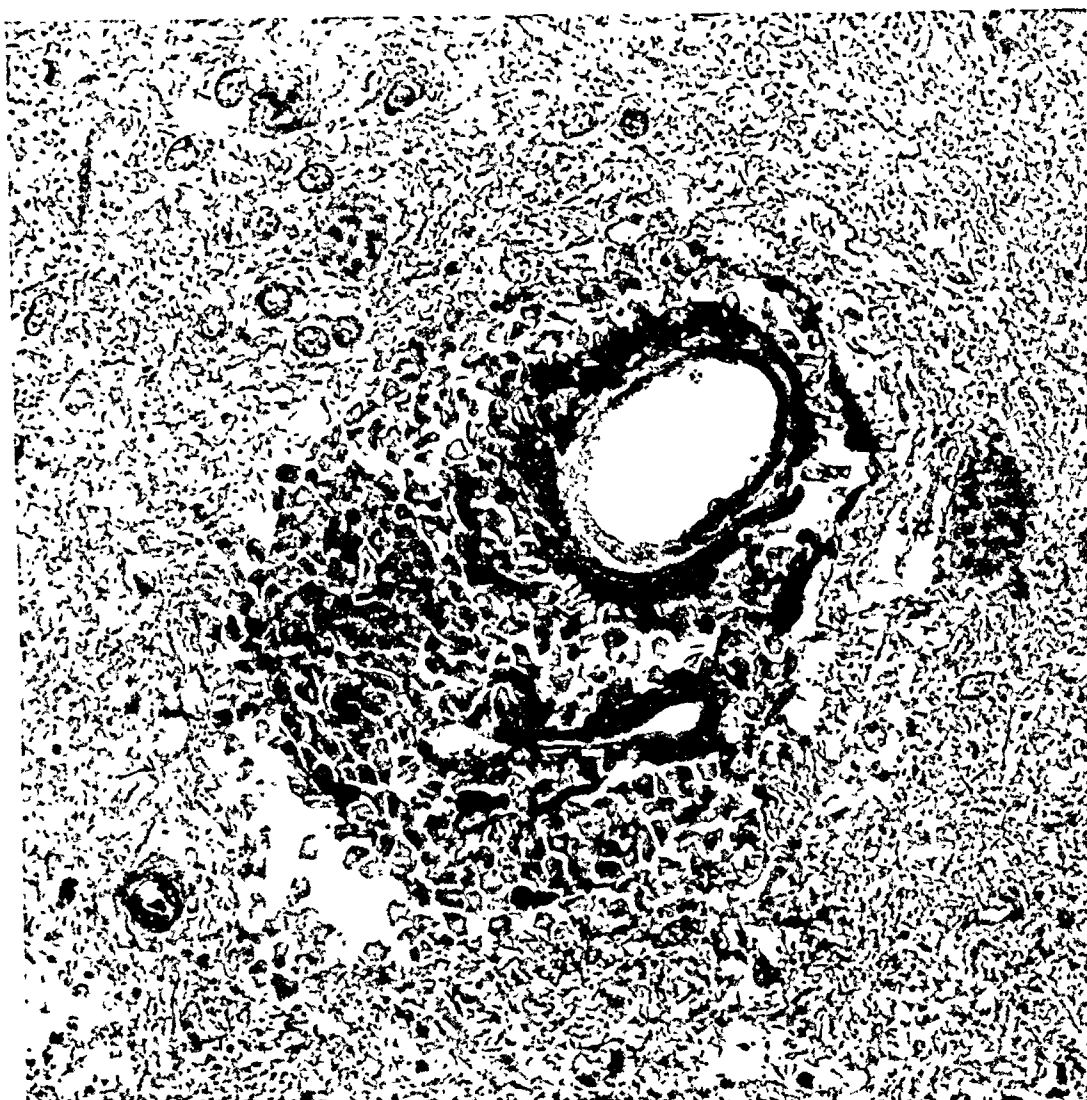


Fig. 7 (case 11).—Section through the lumbar region of the spinal cord showing a perivascular hemorrhage in the gray matter. Masson's trichrome stain.

CASE 9.—This brain was from a white man aged 35, whose illness lasted twelve days. At necropsy the cerebrospinal fluid was said to be opaque, resembling pus. The cerebrum had hemorrhagic spots; likewise, the cerebellum, pons and medulla. Perivascular hemorrhages were found in the subthalamic region, temporal pole, hippocampus (fig. 6), cerebellum, midbrain and occipital lobe. Sections of the paracentral lobule prepared with Loyez' stain showed no loss of myelin. Nissl staining of sections from the paracentral lobule showed no significant changes in

the nerve cells. There was an unusually large amount of fat in the nerve cells of the temporal pole for a man of this age; the microglia cells in this region were normal, although there was some fat about the blood vessels in places. In the temporal pole there was some increase in the subpial neuroglia.

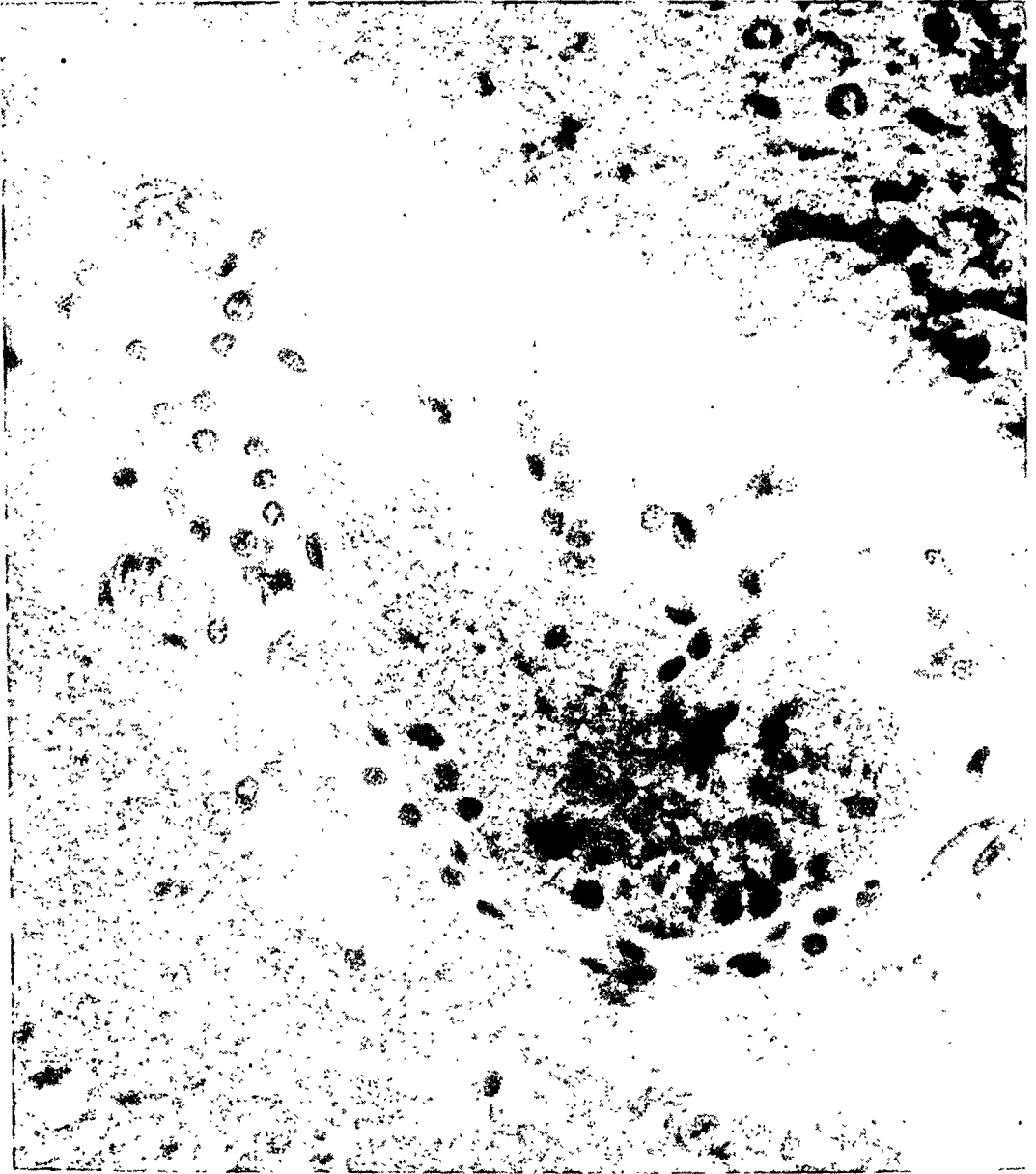


Fig. 8 (case 11).—Section through the lower cervical region of the spinal cord showing some lymphocytes about a blood vessel in the anterior horn of the gray matter and perivascular edema. Hematoxylin and eosin stain.

CASE 10.—This was the brain of a white man aged 26, whose illness lasted five days. At necropsy the meninges were reported as apparently normal. The cerebrum, cerebellum and pons were apparently normal. Perivascular hemorrhages were seen in sections from the temporal lobe and subthalamie region. The microglia

was slightly increased in parts of the temporal lobe. The fat in the nerve cells was normal. The neuroglia was normal.

CASE 11.—The brain was that of a white man aged 41. Sections from the temporal pole, the optic thalamus and the midthoracic and lumbar regions of the spinal cord (fig. 7) showed perivascular hemorrhages. There was some perivascular edema, also some pyknosis of nerve cells, in the superior frontal gyrus. The microglia cells were well stained in parts of the temporal pole, with some transitional forms. Fat was normal in amount in the nerve cells. The neuroglia was slightly increased in the white matter of the temporal pole. Sections through the cervical region of the cord showed some perivascular lymphocytes (fig. 8).

CASE 12.—The brain was from a white man aged 27, whose illness lasted four days and two hours. Perivascular hemorrhages were found in the temporal lobe, hippocampus and subthalamic region. The brain was reported to have no unusual appearance at necropsy. An occasional lymphocyte was seen in the adventitia of a blood vessel here and there. The microglia in the temporal lobe was slightly increased in amount, and a few transitional cells were seen. Fat was increased in the cells and about the vessels slightly. The neuroglia was normal.

CASE 13.—This was the brain of a white boy aged 11, whose illness lasted six days. Perivascular hemorrhages were present in the superior frontal gyrus, the temporal pole, the subthalamic region, the cerebellum, the occipital lobe and the olfactory tract. The microglia in the temporal pole stained fairly well and seemed normal. The fat in the nerve cells was normal. The neuroglia in the temporal pole was normal.

CASE 14.—This brain was from a white man aged 20, whose illness lasted four days and ten hours. Perivascular hemorrhages were present in the temporal lobe, the subthalamic region, the pons, the upper cervical region of the spinal cord and the olfactory tract. An occasional lymphocyte was noted in the adventitia of a few vessels in the optic thalamus. The neuroglia, microglia and fat were normal.

CASE 15.—The brain was that of a white girl aged 12, whose illness lasted five days and ten hours. The pathologist who performed necropsy noted that the contents of the cranial cavity appeared normal. There were perivascular hemorrhages in the inferior temporal gyrus, the midbrain, the medulla and the optic nerve. There was some fibrous arachnoiditis over the paracentral lobule; there was some perivascular edema in the sections from the occipital lobe. The neuroglia and fat were normal, but the microglia was increased in amount.

CASE 16.—This specimen was from a white man aged 23, whose illness lasted four days and fifteen hours. Perivascular hemorrhages were present in the temporal pole, the cerebellum and the occipital pole. There were a few lymphocytes in the perivascular spaces of one or two vessels in the temporal pole. Nissl staining of sections from the occipital pole showed no significant changes. Loyez staining of sections from the occipital pole showed no loss of myelin. The neuroglia in the occipital pole was normal, and the microglia in this region was increased. There was no abnormal amount of fat in the cells of the occipital pole. The Weigert stain for elastic tissue with Van Gieson's stain showed normal vessels in the occipital pole.

CASE 17.—The brain was from a woman whose age was not stated. Her illness lasted about six days. Perivascular hemorrhages were seen in the temporal

lobes, the anterior portion of the striate body and the cerebellum. A few perivascular lymphocytes were seen in sections from the midbrain. The neuroglia, microglia and fat were normal.

CASE 18.—The brain was that of a boy of 4 or 5 years of age, whose illness lasted about four days. Perivascular hemorrhages were seen in the superior frontal

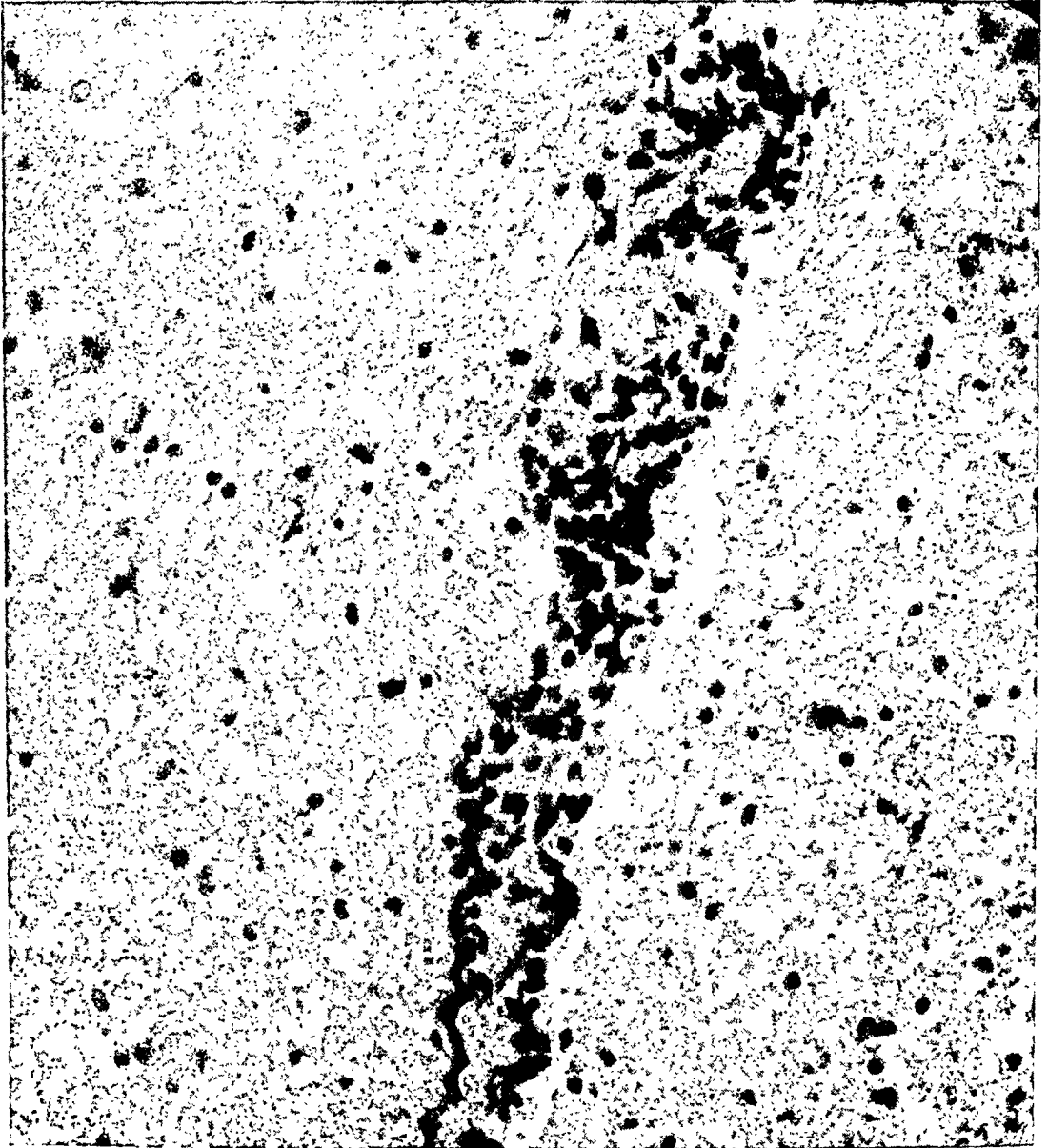


Fig. 9 (case 18).—Section through the optic nerve showing an exudate of lymphocytes about a blood vessel. Hematoxylin and eosin stain.

gyrus, the hippocampus, the midbrain, the subthalamic region, the medulla, the gyrus rectus, the olfactory tract, the optic nerve, the cerebellum and the lenticular nucleus. In the optic nerve there were also a few perivascular lymphocytes (fig. 9). The neuroglia, microglia and fat were normal.

CASE 19.—The brain was from a girl aged 23 months, who died after an illness of four days. Perivascular hemorrhages were present in the hippocampus, the occipital pole, the pons and the temporal lobe. A few perivascular lymphocytes were seen in the hippocampus. The neuroglia was normal. The microglia and fat were slightly increased.

CASE 20.—This brain was from a child of 9, whose illness lasted about four days. A few perivascular hemorrhages were seen in the temporal pole and in the subthalamic region. The neuroglia and fat were normal. The microglia was increased in amount.

In addition to these 20 brains, another was studied in the same way, and although the diagnosis of yellow fever was not confirmed by the findings in the liver, this brain showed lesions exactly like those in the cases in which yellow fever was diagnosed definitely. This brain was from a white man about 35 years of age, who entered the hospital on May 13, 1936, "with a temperature of 36.8 C. (96.9 F.) and a pulse rate of 90 and with the skin and conjunctivae showing jaundice. The patient complained of headache, gastric distress and nausea. Although the patient did not urinate, the bladder was not investigated for the presence of urine. On May 14, the patient presented nervous symptoms, such as picking at the bedclothes. The pupils were dilated. Headache was pronounced. Hemorrhagic vomiting and bleeding from the gums were noted, but neither nose-bleed nor melena was reported. Involuntary urination occurred, but material for examination was not collected. The temperature was subnormal on this day; the pulse was not registered. These symptoms continued on May 16, the day of death." Dr. Fred L. Soper, of Rio de Janeiro, Brazil, furnished these notes on the case after observing the patient in the hospital. He noted in addition that this case seemed to be "typical of severe yellow fever with some involvement of the central nervous system, indicated by mental haziness." Dr. Edgar Cruz, who performed autopsy, stated: "On the basis of the observations at autopsy, I made a diagnosis of yellow fever." Dr. Soper stated that the conditions noted at autopsy included jaundice, remains of black vomit in the buccal cavity and melena in the intestines. In the description of the liver by Dr. Laemmert, however, no evidence of yellow fever was noted. Dr. Soper, in discussing this case in a letter to Dr. E. L. Opie, dated March 2, 1938, stated: This case opens up once more the entire question of the validity of a diagnosis of yellow fever from histologic examination of tissues of the liver. It is known that rhesus monkeys sometimes die of yellow fever without showing what are considered to be the characteristic lesions of yellow fever in the liver. There is, however, available in the municipal laboratory of Rio de Janeiro material from more than 125,000 livers from different parts of South America, mostly Brazil. The collection of this material has been made, as you know, through viscerotomy for the purpose of discovering otherwise undiagnosed outbreaks of yellow fever. Practically all cases in which the liver has been thought to show positive signs or changes arousing suspicion in the laboratory have been investigated in the field, with additional laboratory control, when possible, through attempts to isolate the virus and through determinations of immunity in convalescent persons suspected of having had yellow fever. Likewise, during the same period the laboratory examined a fairly large number of livers from persons whose records describe their condition as suspected of being yellow fever in the field but diagnosed as not that disease after examination of the liver. Whenever possible, these cases have likewise been investigated in the field. As a result of this accumulated experience, my colleagues and I have come to have a high opinion of the examination of liver as a method of detecting otherwise undiagnosable yellow fever. However, we have come to realize that yellow

fever is not obliged to follow the rules laid down for it by any one scientist or group of scientists. For example, there is the possibility, no doubt, that the lesion in the liver is quickly obliterated by regenerative processes, so that persons who die from secondary infections or other causes later than the twelfth or fourteenth day may well fail to obtain a positive diagnosis in the laboratory. Likewise, we have come to recognize that the diagnosis of the lesion in the liver is a subjective process and is not capable of the same objective demonstration as is, for example, the finding of the parasite of malaria in the blood smear. We are not in a position to affirm that yellow fever always produces certain lesions in the liver or to affirm that nothing else can produce the lesions produced by yellow fever. Nevertheless, we continue to perform viscerotomy on a large scale and believe it to be by far the most sensitive indicator of the presence or absence of yellow fever in a given region at the time the investigation is carried out."

The brain in this case showed perivascular hemorrhages in the frontal pole, the tip of the temporal lobe, the midbrain, the cerebellum, the subthalamie region and the medulla. There was perivascular edema in the occipital pole. Sections of the frontal pole treated with the Weigert stain for elastic tissue and counterstained with the Van Gieson stain showed normal vessels. The microglia and neuroglia in the temporal pole were normal. Sections of the frontal pole prepared with the Loyez stain showed no loss of myelin. There was some fat in the perivascular spaces, as well as considerable fat in the nerve cells, in sections from the temporal pole. Nissl staining of sections from the frontal pole showed normal nerve cells. My associates and I considered this to be one of the most typical of the cases of encephalopathy produced by yellow fever before we learned that study of the liver did not confirm the clinical diagnosis.

A table is appended to show the sites of hemorrhages in the 20 brains. The table also shows what other regions of the brain were examined in each case in which no hemorrhages were found. As I have noted in an earlier part of this paper, the hemorrhagic condition in the brain and spinal cord in yellow fever resembles very much what my associates and I and what others have seen in alcoholic encephalopathy. However, in yellow fever the condition is more severe and probably would be fatal even if no other lesions were present in the liver or other organs. Apparently the hemorrhages are of short duration, for there is usually no notable reaction on the part of the microglia or astrocytes, and few phagocytes are encountered. It is probable that these hemorrhages are not more than a day or two old.

Arsphenamine will occasionally cause a fatal hemorrhagic condition of the brain, with the brunt of the lesion being borne by the lenticular nucleus. Here, of course, there is damage to the blood vessels by the toxin, with resulting hemorrhage. Other poisons, such as cyanides, mercury, manganese, lead and phosphorus, may cause minute hemorrhages in the brain along with other changes. Carbon monoxide poisoning may cause hemorrhages in the brain, but in addition there are necrosis of the globus pallidus and thrombosis of small vessels. Snake venom and many other poisons, some used in industry, may cause small hemorrhages in the brain. We have just examined

Chart Showing Sites of Hemorrhage in the Brain in Twenty Cases of Yellow Fever

Case	Periventricular and Sub-thalamic Region at Level of Mamillary Body										Cerebellum		Spinal Cord	Olfactory Tract	Optic Nerve	Other Cranial Nerves
	Frontal Pole	Superior Frontal Gyrus	Para-central Lobule	Temporal Lobe	Temporal Pole	Hippocampus	Occipital Lobe	Occipital Pole	Insula	Mamillary Body	Lenticular Nucleus	Mid-brain	Pons	Medulla		
1	○	○	+		+	+	+	+	+	+	+	○	+	+	+	..
2	+	..	○	..	+	..	○	○	○	○	Badly decomposed	..
3	..	+	+	+	..	+	+	+	+	+
4	+	+	+	+	+	○	+	○	..	+	○
5	+	○	+	..	+	..	○	○	..	○	..	○	○
6	+	○	+	+	+	○	○	○	○	+	○	○	+	+
7	..	○	+	..	+	○	..	+	○	+	○	○	○	+
8	..	+	+	+	..	○	..	+	○	+	..	+	..	+	+	..
9	○	..	+	+	+	+	..	+
10	○	+	..	○	○	+	○	○	..	○
11	○	○	+	○	○	+	○	..	+
12	..	○	..	+	..	+	+	○	○	○	○ 3d
13	..	+	+	○	+	+	..	○	+	+ 2d 5th 3d 7th 4th 8th
14	..	○	..	+	..	○	..	○	..	+	+	+	+	..
15	○	..	+	..	○	+
16	..	○	+	○	..	+	○	..	○	○
17	○	○	..	+	+	○	○	+	○	+	○	○	..
18	..	+	+	+	+	+	+	+	+	3d 5th
19	..	○	..	+	..	+	..	+	..	○	○	..	○	○	○	○ 3d
20	○	+	○	..	+	..	○	○	○

the brain of a dog poisoned with metrazol, a convulsant drug used recently in the treatment of dementia praecox. We found perivascular hemorrhages. In cases of anoxemia of the brain small hemorrhages are common about the vessels. In cases of stasis of blood flow in the smaller vessels of the brain due to leukemia hemorrhage of the brain is not uncommon. In cases of acute anterior poliomyelitis small hemorrhages are usually encountered in the gray matter of the spinal cord as well as a perivascular exudation of lymphocytes and even of polymorphonuclear cells. In addition, however, there can be seen a specific effect of the virus on the nerve cells. In many cases of lethargic encephalitis large or small hemorrhages may be found in the lower parts of the brain. We have observed, also, perivascular hemorrhage in cases of pertussis.

In a true virus infection of the nervous system, lasting several days, there would undoubtedly be ample evidence of the infection in the brain in the form of lymphocytic accumulations in the Virchow-Robin spaces, in addition to other pathologic changes in the nerve cells, microglia, astrocytes and even the myelin sheaths at times. In these cases of yellow fever the hemorrhages were the most significant change discovered in the nervous system and were apparently a terminal event. They cannot be considered as evidence of neurotropism of the virus of yellow fever in man.

Most of these 20 cases of yellow fever occurred in the states of Paraná or Minas-Geraes in Brazil in January, February, March and April 1936. The average duration of illness was a little over six days, and the average age of the patients was 29 years. The youngest patient was about 5 years of age, and the oldest was 62.

The illness clinically was typical of yellow fever, beginning with headache and pains in the back. There were fever and much thirst, with epigastric pain, enlargement of the liver, some jaundice, often photophobia and congestion of the eyes and face. Black vomit and melena occurred in most instances, with albuminuria, oliguria and later anuria. Delirium and coma were present in some a day or so before death, and convulsions were present in a single case. Nervousness was noted in several.

The literature consulted threw little light on the question of the involvement of the nervous system in man. Jakob, Fialho and Villela^{1a} reported on 14 cases in man in Rio de Janeiro, Brazil. No gross pathologic lesion was reported, but microscopically there was small cell infiltration of the leptomeninges, with macrophages containing blood pigment, also some edema of the pia. There was severe fatty degen-

1a. Jakob, A.; Fialho, A., and Villela, E. L.: *Deutsche Ztschr. f. Nervenhe.* 3:111, 1929.

eration of the nerve cells in the cortex and striatum. The authors noted proliferation of glia cells and some chromatolysis of nerve cells, with focal atrophies in the cortex and other tissues.

Stefanopoulo and Mollaret² reported observation of hemiplegia and optic neuritis in a case of yellow fever. The patient, however, recovered, so it is not known what the nature of the lesions in the brain was.

Findlay and Stern³ noted ptosis of the left eyelid and partial facial paralysis in a patient who suffered from a severe but typical attack of yellow fever in Northern Nigeria.

Apparently in mice, monkeys and other animals true encephalitis may develop following inoculation with the virus of yellow fever under certain conditions. In the monkey (*Rhesus*) encephalitic symptoms are associated with microglial proliferation, perivascular infiltration and intranuclear inclusions (Findlay and Stern³). These authors concluded that there is evidence that neurotropic potentialities are inherent in the ordinary strain of the virus. Other authors have reported encephalitis in *Macacus rhesus* by inoculation of viscerotropic yellow fever (Goodpasture⁴; Penna⁵). Encephalomyelitis following vaccination against yellow fever has been reported by Lhermitte and Fribourg-Blanc⁶ and by Dezeit⁷). This, however, is quite another matter and is comparable to encephalitis following vaccination against smallpox. In the fatal case reported by Lhermitte and Fribourg-Blanc the patient died about fifteen months after vaccination. The lesions in this case resemble those seen in disseminated sclerosis.

SUMMARY

Preliminary studies were made on different regions of the brain in 14 cases of yellow fever in man. In 20 other cases of yellow fever in man the whole brain was available for study and in 3 of these cases the spinal cord as well. A detailed examination of the brains in these 20 cases is reported in this paper.

The chief lesion found in all of the brains studied was perivascular hemorrhage. These hemorrhages were most frequently found in the subthalamic and periventricular region at the level of the mamillary bodies. The temporal pole was next most involved and the cerebellum only slightly less so.

2. Stefanopoulo, G. J., and Mollaret, P.: *Bull. et mém. Soc. méd. d. hôp. de Paris* **50**:1463, 1934.

3. Findlay, G. M., and Stern, R. O.: *J. Path. & Bact.* **41**:431, 1935.

4. Goodpasture, E. W.: *Am. J. Path.* **8**:137, 1932.

5. Penna, H. A.: *Am. J. Trop. Med.* **16**:331, 1936.

6. Lhermitte, J., and Fribourg-Blanc: *Rev. neurol.* **65**:391, 1936.

7. Dezeit, G.: *Bull. Soc. path. exot.* **30**:253, 1937.

Perivascular lymphocytic exudate was noted in 9 cases and with the exception of a single case this was slight.

Changes in the nerve cells were insignificant, and no inclusion bodies were seen.

Reactive changes in the microglia and astrocytes were slight.

From this study it is concluded that there is no definite evidence of neurotropism on the part of the virus of yellow fever in this series of cases.

PRIMARY SYSTEMIC AMYLOIDOSIS

INVOLVEMENT OF CARDIAC VALVES, JOINTS AND BONES, WITH
PATHOLOGIC FRACTURE OF THE FEMUR

SIMON KOLETSKY, M.D.

AND

ROBERT M. STECHER, M.D.

CLEVELAND

Amyloidosis secondary to chronic suppurative disease or tuberculosis is relatively common. In contrast, the primary form of amyloid disease is distinctly rare. Yet within recent years primary systemic amyloidosis has become a recognized entity. A number of those who have reported cases of this disease have emphasized (1) the absence of known etiologic factors and (2) the tendency to involve smooth and skeletal muscle while organs such as the liver and spleen, usually affected in secondary amyloidosis, are uninvolved.

The following case of primary systemic amyloid disease is reported because of extensive involvement of the cardiac valves and of the joints and bones, with pathologic fracture of the femur.

REPORT OF A CASE

A 61 year old white woman was admitted to the Cleveland City Hospital Sept. 11, 1935, with complaints of fracture of the left hip and rheumatism. In 1924 she noticed for the first time swelling and stiffness of the fingers of the right hand. This was accompanied by tingling and burning sensations, which persisted for five years and then stopped. Eight years before admission she noted swelling and stiffness of the right wrist. After that all her joints gradually became stiff and swollen. This resulted in moderate disability, although there was no pain. During the past two years her tongue grew larger. About three months prior to admission she experienced sudden pain in the left hip while walking and fell down. She was put to bed and was unable to arise because of severe pain. Hospitalization was advised by a physician because of a fracture of the hip.

The past history revealed that the patient had been in good general health except for the disabilities mentioned. Her diet had always included a large amount of meat.

The temperature was 36.8 C. (97.9 F.); the pulse rate, 96; the respiratory rate, 21, and the blood pressure, 136 systolic and 88 diastolic. The patient was well nourished and not severely ill. The skin was smooth, glassy and free from wrinkles. The subcutaneous tissue and skeletal musculature throughout the body were firm. There was no icterus or superficial lymphadenopathy. The pupils

From the departments of pathology and medicine, Cleveland City Hospital and Western Reserve University.

reacted to light and accommodation. The fundi showed a slight degree of arteriosclerosis.

The tongue was enlarged and showed small nodules on the dorsum. On the inner aspects of the lips there were firm, elevated nontender nodules 1 cm. in diameter and of a grayish white appearance.

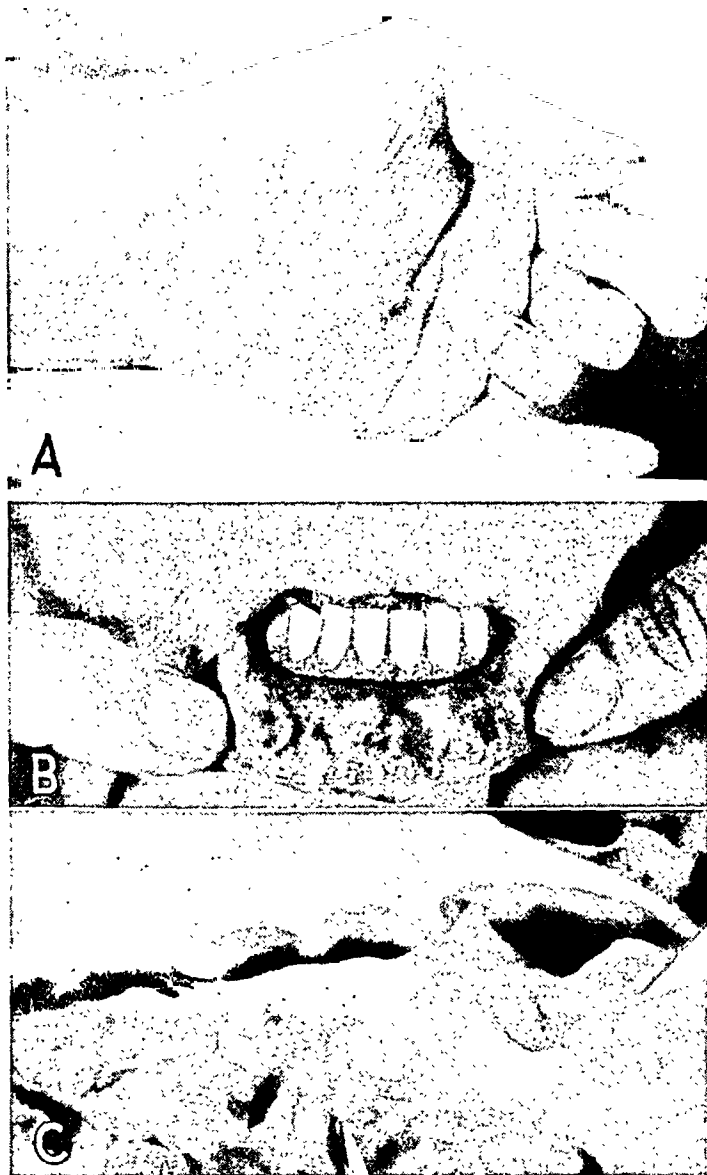


Fig. 1.—*A*, swelling and nodularity of the left wrist and palm; *B*, nodular lesions of the lower lip; *C*, amyloid involvement of the aortic valve.

The heart was not enlarged. The rate was normal and the rhythm regular. There was a loud blowing systolic murmur at the apex. The second sound at the base was harsh. The lungs and abdomen were normal. The liver and spleen were not palpable. Pelvic examination revealed firm nodular lesions from 1 to 2 cm. in diameter on the posterior vaginal wall just within the introitus.

All the joints were irregularly enlarged, firm to palpation and not tender. Much of the swelling and irregularity was apparently due to firm nodular masses involving the tendon sheaths and ligaments of the joints. Both limitation and rigidity of motion were present throughout. The shoulders seemed to be padded, and their bony markings were obscured. The wrists and elbows were irregularly swollen on the dorsal and volar aspects. In the antecubital spaces there were firm, elevated nodules 3 cm. in diameter, probably attached to the tendons and muscles. The left hip and thigh were diffusely enlarged. There was a firm immobile non-tender mass extending down from the crest of the ilium and merging with the structures of the thigh.

The fingers of both hands were rigid and immobile except for a slight degree of passive flexion. The palms showed many subcutaneous nodular swellings. Both the thenar and the hypothenar eminences were obscured. On the dorsal aspect of the left hand there were nodules 1 cm. in diameter attached to the tendons.

There was no evident involvement of the vertebral column.

The urine was normal. An examination for Bence Jones protein was not made. The red blood cell count was 3,900,000; the hemoglobin content, 55 per cent; the white blood cell count, 6,200. The Wassermann reaction of the blood was negative. The serum phosphorus was 4.2 mg. and the serum calcium 9.4 mg. per hundred cubic centimeters. The phosphatase activity was 9.5 units. The serum cholesterol was 242 and 235 mg. per hundred cubic centimeters. Repeated examinations of the blood for urea nitrogen gave values within normal limits. Dextrose tolerance tests gave results as follows: during fasting, 98 mg. per hundred cubic centimeters of blood; at one half hour after administration of dextrose, 186 mg.; at one hour, 220 mg.; at two hours, 178 mg.; at three hours, 184 mg. The icterus index was 5. The blood uric acid was 4.1 and 4.3 mg. per hundred cubic centimeters; the total blood fat was 1.17 mg. per hundred cubic centimeters.

The congo red test showed on the first specimen 100 per cent, and on the second specimen 133 per cent, of the dye present (hemolysis).

The roentgenologic reports Sept. 16 to 17, 1935, were as follows: The left hip showed a fracture through the neck of the femur, with considerable fragmentation and absorption of the bone and slight upward displacement of the shaft. This fracture did not have the appearance of a recent one. Both hands, the left knee and the right shoulder showed generalized demineralization of all the bones. There appeared to be fragmentation about the greater tuberosity and a slight downward displacement of the head of the humerus. No other pathologic process was noted in these areas. The lumbar region of the spinal column showed similar demineralization of bone. Otherwise there was no significant change. There was no definite pathologic process in the right femur. A roentgenogram of the chest showed the cardiac shadow to be slightly increased in its transverse diameter, indicating cardiac hypertrophy and dilatation. The lungs were normal.

On admission the diagnosis of a pathologic fracture of the neck of the left femur was made. The underlying disease of the bone, as well as the nature of the involvement of the joints, was not clear.

The patient remained in the hospital for almost two and a half years before she died. For most of this period the clinical picture was puzzling. The joints, although swollen and deformed, were roentgenologically normal. No form of arthritis adequately explained the extensive involvement of the tendons. The diagnosis of gout was eliminated because of the lack of pain, the absence of tophi and the normal level of the uric acid in the blood. Although the patient was not diabetic, xanthoma tuberosum multiplex was considered because of the involvement of tendons, the subcutaneous and submucous nodules and the erosion of bone. However, the absence of cholesterol in the biopsy material excluded this possibility.

The first biopsy specimens consisted of a segment of extensor tendon from the right wrist and some small firm translucent masses from the joint space. Microscopically, the connective tissue of the tendon was largely replaced by homogeneous hyaline material. There was no inflammation, and no foam cells were

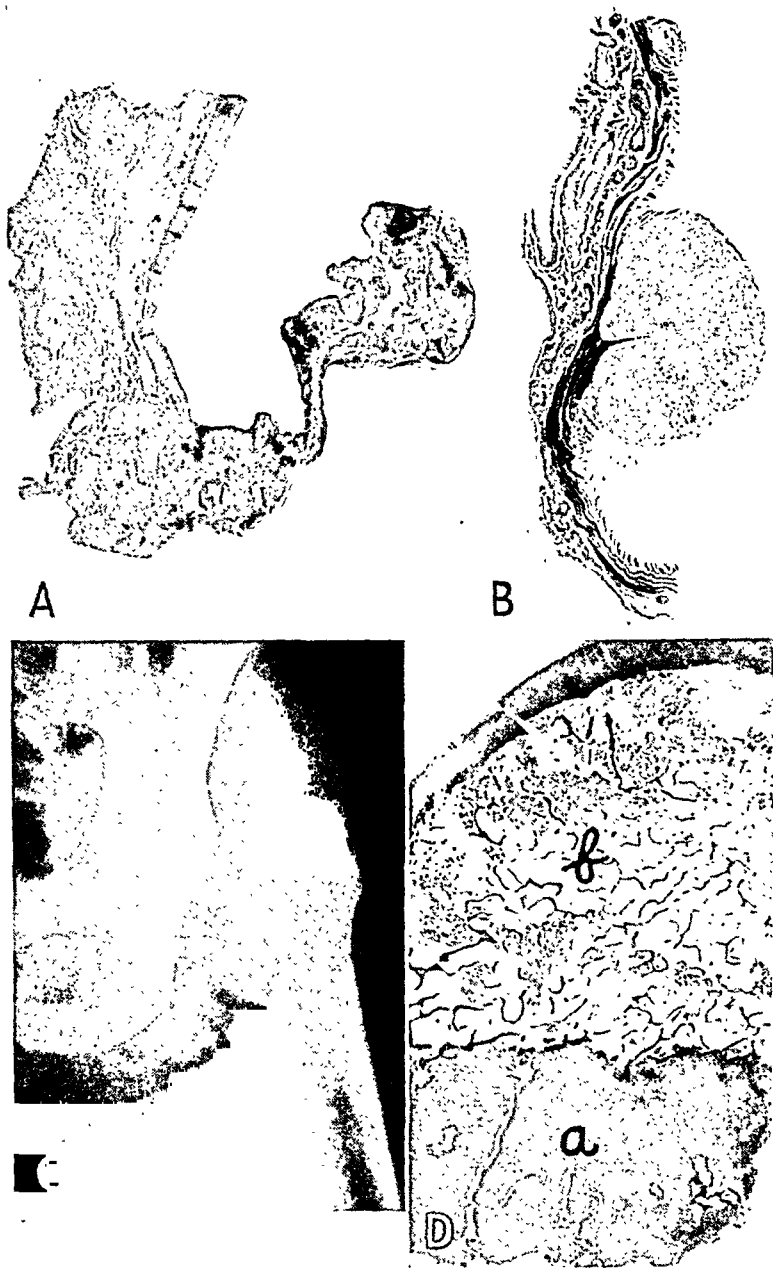


Fig. 2.—*A*, microscopic section through the midportion of the aortic cusp; $\times 3$: amyloid involvement of pericardial wedge, ring and free portion of valve. *B*, amyloid nodules of the small intestine as shown in a microscopic section; $\times 3.87$. *C*, pathologic fracture of the left femur. *D*, microscopic section of the head of the left femur showing (*a*) amyloid extending to the line of fracture and (*b*) a spongy portion of bone; $\times 2.32$.

seen. With polarized light no anisotropic substance was found. The diagnosis was chronic productive and degenerative tenosynovitis. A second biopsy specimen taken from the right shoulder consisted of fragments of firm tissue of cartilaginous consistency, and the diagnosis was hyalinization of connective tissue. Later, tendon sheaths were removed from both wrists. Microscopic sections showed replacement of the tendon by a hyaline material which gave positive reactions for amyloid with methyl violet and congo red. There was no anisotropic substance. A diagnosis of chronic tenositis with amyloid change was made. Following the recognition of amyloid in the third biopsy specimen, the dermatologic consultants, who had been interested in the lesions of the tongue and lips, made a diagnosis of primary systemic amyloidosis.¹ Stains for amyloid in the earlier biopsy specimens were then found to be positive.

During the entire period of hospitalization the patient's condition remained stationary and, although largely bedridden, she was in fair health and had no additional complaints. With rest in bed, the pain in the left hip diminished as motion of the joint decreased and disappeared when ankylosis was complete. Subsequent roentgen examinations showed no appreciable change in appearance over that at admission. A subtrochanteric osteotomy was advised by the orthopedic consultant but was refused by the patient. She was given physical therapy and, with massage and gentle manipulation, gained some use of all her extremities. With a minimal amount of help, she was able to walk short distances on crutches and even resumed slight bearing of weight on the left leg.

A sudden attack of pain and blindness in the left eye occurred in December 1935, which was thought by the consultant to be due to embolism or spasm of the central artery. Following this episode there was permanent impairment of vision in the left eye.

In February 1938 the patient, after having been afebrile, began to have fever, cough and dulness to percussion over the entire right side of the chest. She died February 13 of confluent bronchopneumonia, after an illness of eight days.

The final clinical diagnosis was: primary systemic amyloidosis and terminal bronchopneumonia.

Autopsy.—A postmortem examination was made seven hours after death. There was no icterus or lymphadenopathy. The inner aspects of the lips and the tongue were as described already. The left lower extremity was 6 cm. shorter than the right. The skin throughout the body was smooth and glassy but on section showed no significant change in the epidermis or corium. The subcutaneous fat was firm and had a peculiar pale yellow color.

The skeletal muscles of the thoracic and abdominal walls were tense and rigid, and their fibers were extensively replaced by confluent nodular masses of translucent pearly gray lardaceous material. Gross tests of this material for amyloid by means of the iodine and sulfuric acid stain were strongly positive.

Externally all the joints were swollen and irregular in contour. The left elbow and left hip joints were selected for examination.

The entire articular capsule and synovial membrane of the left elbow joint were replaced by firm lardaceous grayish yellow tissue which extended into the joint space and filled the olecranon, coronoid and radial fossae. At the sites of attachment of the articular capsule to bone there was direct invasion by this tissue of the humerus, the olecranon and coronoid process of the ulna and the head of the radius.

1. The patient was presented at a meeting of the Cleveland Dermatological Society, March 25, 1937 (*Arch. Dermat. & Syph.* **37**:330, 1938), as having primary systemic amyloidosis.

The left hip joint was partly encased in a huge solid mass of light yellow lardaceous tissue, which measured 10 by 5 by 4 cm. This tissue extended through the articular capsule to fill the joint space and had invaded the entire neck of the femur. There was a complete fracture through the middle of the neck, with separation of the fragments. The surface of the fracture showed numerous irregular tags of lardlike material. The articular surfaces of the joint were uninvolved except for the fovea capitis of the femur which was invaded by extension from the ligamentum teres. All the tendons adjacent to both the elbow and the hip joint were extensively replaced by firm translucent lardaceous tissue. The material in



Fig. 3.—Left femur. The upper arrow points to the line of fracture; the lower, to a mass of amyloid.

the joints, bones and tendons gave strongly positive reactions for amyloid with iodine and sulfuric acid.

The heart weighed 400 Gm. There were numerous firm raised pearly gray nodules, measuring 1 to 2 mm. in diameter, in the epicardium of the atria and in that of the base of the ventricles. The left atrial chamber was enlarged and had a thickened, rigid, leathery wall which did not collapse. Its muscle layer and endocardium were extensively replaced by firm grayish white tissue, and the entire endocardium presented a grayish yellow glistening nodular surface. The changes in the right atrium were similar but less extensive. The left ventricular chamber was small, while the right was considerably dilated. Both ventricles presented a slightly hypertrophic brownish red myocardium, which showed no fibrosis. There were occasional small grayish white deposits of amyloid, especially in a sub-endocardial position. The coronary arteries revealed no significant change.

All four cardiac valves showed nodular deposits of amyloid. The involvement of the mitral and aortic valves was particularly extensive. Numerous glistening pearly gray beadlike nodules were deposited in the base and free portions of the mitral leaflets, especially on their atrial surfaces near the free margins. As a result the leaflets were thickened, rigid and virtually immobile. Although there was no fusion of the commissures, the orifice of the valve was fixed in a markedly stenotic position. The chordae tendineae were slightly thickened but discrete. There were numerous nodular deposits in the free portions of the tricuspid leaflets. The aortic valve was severely deformed by the amyloid infiltration, and its cusps were thickened, retracted and immobile. Both the atrial and ventricular surfaces of the cusps, as well as the sinuses of Valsalva and the subvalvular endocardium, were studded with discrete and confluent hard pearly white nodular excrescences. Although the commissures were not fused, the valvular orifice was definitely stenotic. The pulmonic valve was the least affected, showing only a few nodular deposits on the ventricular aspects of the cusps.

The aorta and pulmonary arteries were the seat of moderate arteriosclerotic change but were otherwise not unusual.

The lungs showed confluent bronchopneumonia, which was especially severe in the lower lobes. The crepitant portions were grayish brown and were firm and indurated to palpation. Moderate arteriosclerotic change of the small pulmonary arteries was present. The bronchi presented a hyperemic brownish red granular mucosa but their lumens contained no exudate. The hilar lymph nodes were small and showed nothing unusual on section.

The liver and spleen weighed 1,250 Gm. and 150 Gm., respectively, and were the seat of passive hyperemia. Otherwise they showed nothing unusual.

No significant changes were noted in the gallbladder and biliary ducts, pancreas, adrenals, urinary tract, pelvic organs, breasts, thyroid and parathyroids, diaphragm, esophagus, stomach, duodenum and brain. There was no abdominal lymphadenopathy.

The posterior wall of the vagina just within the introitus showed several small elevated nodular masses of firm grayish white tissue from 1 to 2 cm. in diameter.

The midportion of the jejunum and the large intestine from the ascending colon to the sigmoid showed in their walls numerous discrete pearly gray nodules 0.5 to 5 mm. in diameter. These lesions cut with resistance. Grossly they appeared to be confined to the muscle layer and were covered by intact mucosal and serosal surfaces. Gross tests for amyloid were positive.

The ribs and vertebrae were intact and presented the usual red marrow. The marrow of the upper half of the left femur was hyperplastic, friable and pale red.

Microscopic Examination.—The histologic preparations were stained by the hematoxylin and eosin, Van Gieson and azocarmine methods. Both fresh tissue and tissue fixed in solution of formaldehyde were examined for amyloid with congo red, methyl green and iodine-sulfuric acid stains.

Both ventricles of the heart revealed hypertrophy of their fibers. The small blood vessels, especially the arteries, showed subendothelial and medial deposition of an eosin-staining homogeneous material, which was positive for amyloid. The muscle was occasionally interrupted by nodular deposits of the same substance. The involvement of the left atrium was extensive. In one area of deposit in the endocardium there was progressive transformation of amyloid through the stage of cartilage to bone. The bony trabeculae enclosed a fatty marrow, which showed a few hemopoietic cells.

Analysis of Twenty-Two Cases of Primary Systemic Amyloidosis as Recorded in the Literature

Authors	Year	Age	Sex	Symptoms	Clinical Diagnosis	Total Duration of Disease	Cause of Death	Distribution of Amyloid at Autopsy
Wild ^{sa}	1886	56	♀	History incomplete	Pulmonary emphysema, cardiac insuffi- ciency	Not known	Erysipelas	Tongue, heart, heart valves, gastrointes- tinal tract, lymph nodes, bladder, lungs, pericardium, peritoneum
Steinhaus ^{sb}	1902	40	♂	Intestinal hemorrhages; dyspnea; vomiting; ob- stipation	Carcinoma of pylorus	6 mo.	Shock	Heart, stomach, pylorus, intestine
Ritter ^{sc}	1908	50	♂	Pain in sacral region and extremities; swelling of tongue, dysphonia, and dysphagia; constipation	Carcinoma of tongue, inanition	2 yr.	Bronchopneu- monia, cardiac insufficiency	Tongue, stomach, intestine, ? heart
Beneke st	1922			Data not available				Heart, tendons, ligaments and capsules of joints, large veins, pulmonary arteries and veins
Königstein ^s	1925	60	♂	Pain in shoulders, arms and hands; weakness; swelling of tongue; dysphonia and dys- phagia; constipation and diarrhea	Generalized amyloidosis	2 yr.	Ileus, pneu- monia	Tongue, heart, skin, skeletal muscles, gastrointestinal tract, adipose tissue, bladder, urethra, corpus cavernosum of penis, superior vena cava
Lubarsch ^{sa} (3 cases)...	1927	53	♂	Headaches and dizziness; lameness	Myotonia, scleroderma, carcinoma of tongue	8 yr.	Pneumonia, emphysema	Tongue, heart, skin, skeletal muscles, gas- trointestinal tract, lymph nodes, bladder, prostate, seminal vesicles, testes, epididy- mides, piarynx, esophagus, lungs, pleurae, diaphragm, peritoneum, dura, adrenals, capsule and tendons of knee and hip joints
	1927	66	♀	Purpura of skin; headaches; dyspnea	Thrombopenic purpura, cysto- pyelitis, bronchitis	14 mo.	Cardiac insuffi- ciency	Tongue, heart, skin, gastrointestinal tract, esophagus, lungs
	1927	45	♂	Hematemesis; anorexia; con- stipation, weakness and pain after meals	Carcinoma of stomach	1 yr.	Death followed laparotomy	Heart, gastrointestinal tract, lungs, lymph nodes (thoracic, abdominal and inguinal), mesentery, prostate, seminal vesicles, testes, epididymides, spleen (pyloric ulcers)
Picchini and Fabris ^{se}	1930	51	♀	Swelling of tongue, dysphonia and dysphagia; dyspnea; swelling of external genitalia	Scleromegaly with multiple hemorrhages and lymph- adenopathy	3 yr.	Death followed tracheotomy to relieve dyspnea	Tongue, heart, lips, palate, larynx, trachea, muscles of neck
Warren ^{sh}	1930	54	♀	Canker sores of tongue; swell- ing of tongue and cheeks	Leukoplakia of tongue	20 mo.	Bronchopneu- monia	Tongue, heart, skeletal muscles, gastro- intestinal tract, diaphragm, cheeks, esopha- gus, gallbladder, urinary bladder, uterus, arteries and veins
Pick ^{sl}	1931	54	♂	Disturbances of motion; swell- ing of tongue and dysphagia	Myotonia, scleroderma, carcinoma of tongue	Not known		Tongue, heart, gastrointestinal tract, lungs, esophagus, pharynx, serosal surfaces

Gottroff ¹	1932	16	♀	Difficulty in standing, walking; swelling of tongue, dysphagia and dysphonia; constipation	Systemic amyloid disease	3 yr.	Tongue, skin, skeletal muscles, gastrointestinal tract (clinical evidence)
Gerstel ²	1932	52	♀	Swelling of tongue and dysphagia; dyspnea; diarrhea - melaena; constipation; weakness	Carcinoma of floor of mouth, intestinal paralysis	2 yr.	Cardiac failure	Tongue, heart (subpericardium), gastrointestinal tract, skeletal muscles (neck and pharyngeal region), mouth, soft palate, pharynx, esophagus, pericardiacal fibroadipose tissue, skin of neck, mesentery, diaphragm, adventitia of large vessels
Mollow and Lebell ³	1932	60	♂	Abdominal pain, meteorism, constipation; swelling of tongue, dysphonia and dysphagia; difficulty in walking	Paralysis of larynx, hypertonia of muscles, stenosis of sigmoid colon	16 mo.	Cardiac failure	Tongue, heart, gastrointestinal tract, skeletal muscles, skin, diaphragm
Von Bonsdorff ⁴	1933	51	♂	Stiffness of tongue; large tumors about joints; weakness	Local amyloid disease	1½ yr.	Bronchopneumonia	Tongue, mouth, tendons, joints, bones, lymph nodes (axillary)
Strauss ⁵	1933	72	♂	Tendency to stoop; dyspnea; cervical adenopathy	Carcinoma of lung, carcinomatosis	1 yr.	Not stated	Heart (epicardium), pericardium, lungs, lymph nodes (universal), connective tissue of mediastinum, appendix, periaortic fibroadipose tissue
Michelson and Lynch ⁶	1934	51	♂	Pain in lumbar region and joints, limitation of motion; swelling of tongue, dysphonia; constipation	Systematized amyloidosis	18 mo.	Intestinal hemorrhage, ileus	Skin, tongue, buccal and anal mucosa (clinical evidence)
Gaupp ⁷	1934	53	♀	Weakness, fatigue, difficulty in walking	Not given	2 yr.	Patient died suddenly	Tongue, heart, skin, cervical lymph nodes, aorta, pulmonary arteries, skeletal muscles, uterus, kidneys
Perla and Gross ^{2a}	1935	53	♀	Dyspnea, cough, pain in chest, loss of weight, weakness	?Carcinoma of lung, congestive heart failure	Several months	Sudden circulatory collapse	Tongue, heart, gastrointestinal tract, lungs, diaphragm, uterus, kidneys
Reimann and others ⁸	1935	41	♀	Pain in extremities; swelling of tongue and submental region, dysphagia, dysphonia, dyspnea; amenorrhea	Amyloid disease	2 yr.	Peritonitis	Tongue, heart, lungs, esophagus, pelvic organs (clitoris, vulva, vagina, uterus), mediastinum
Weber and others ^{8a} ...	1937	48	♀	Pain in fingers and shoulders; swelling of tongue and neck, dysphonia; fatigue; intermittent claudication	Systematized atypical amyloidosis	2 yr. 1 mo.	Patient discharged from hospital after resection of tongue	No autopsy
DeNavasquez and Treble ^{3b}	1938	36	♂	Diarrhea; muscular weakness; dyspnea; dizziness on exertion	Berberi, chronic enteritis	2 yr. 2 mo.	Streptococcal pharyngitis, cardiac failure	Heart, intestines, posterior root and sympathetic ganglia, peripheral nerves, thyroid, adrenals, testes, spleen, arterioles (generalized)
Haenisch ⁴	1938	58	♀	Carcinoma of bladder, papillomatosis of bladder; myocardial disease, bronchopneumonia	...	Cardiac failure	Heart, tongue, urinary bladder
Koletsky and Stecher	1938	61	♀	Swelling of joints, limitation of motion; fracture of left hip; swelling of tongue	Primary systemic amyloidosis	11 yr.	Bronchopneumonia	Tongue, heart, heart valves, skeletal muscles, intestines, joints, bones, tendons, vagina, lips

The pericardial wedge and ring of the aortic valve showed numerous nodular hyaline masses. All the layers of the free portion, especially the fibrosa, were likewise involved, and the tip of the valve showed confluent deposits of this substance. The elastic fibers were essentially intact, although frequently compressed and distorted by the amyloid. There was no inflammatory reaction, and only vessels of the sinusoidal type free from amyloid disease were present. The mitral ring and valve showed similar extensive nodular deposits of amyloid. There was no vascularity or inflammation.

The lungs showed areas of edema and bronchopneumonia. The alveolar walls were thick, owing to an increase in fibrous tissue, and numerous "heart failure" cells were present within the alveolar spaces. The walls of the small arteries were distinctly thickened. Occasionally a small artery or vein showed subendothelial deposition of amyloid.

In the liver and spleen severe passive hyperemia was observed. No amyloid involvement was found.

The vagina showed nodular hyaline masses in the tunica propria of the mucosa and in the underlying muscle. The small arteries in the neighborhood of these deposits showed amyloid infiltration. There was a slight amount of hemorrhage.

In the intestines the longitudinal layer of muscle was interrupted by nodular masses of homogeneous acidophilic material. The circular layer was compressed but otherwise uninvolved. There was irregular replacement of the muscularis mucosae by amyloid, which extended into the tunica propria. The small arteries, particularly those of the submucosa, were the seat of severe amyloid deposition. In some sections the blood vessels showed extensive infiltration while the muscle was free from disease.

The epidermis and corium were normal. Nodular deposits of amyloid were present deep in the subcutaneous tissue adjacent to the underlying fascia and muscle. Several nodules showed areas of transformation of amyloid into cartilage.

Deposits of acidophilic homogeneous material were found in the connective tissue of the tunica propria and underlying muscle of the lip.

The tumor mass of the left hip was composed of confluent lobulated masses of amyloid. Some of the lobules revealed blue-staining areas which suggested early cartilage formation. The vessels consisted of endothelial slits surrounded by amyloid.

The fibers of the skeletal muscle were spread apart and in some areas replaced by diffuse nodular deposits of amyloid. Most of the muscle cells appeared normal even when surrounded and compressed by this substance. Some were atrophic as a result of pressure. In several areas bands of amyloid in the interstitial connective tissue seemed to invaginate and possibly invade the fibers. The small arteries in the interstitial stroma showed extensive amyloid infiltration of their walls.

At the line of fracture of the neck of the left femur the bone was completely replaced by amyloid. The boundary between amyloid and the uninvolved cancellous bone of the head of the femur was sharp. The latter showed fatty marrow with islands of hemopoiesis. Within the amyloid tissue were several small foci of bone formation similar to that in the left atrium. Marrow from the upper portion of the left femur showed myeloid hyperplasia and no amyloid. Histologic section of a vertebra also showed myeloid hyperplasia. Some of the small arteries were the seat of severe amyloid deposition, but their lumens were not reduced. The reticulum and bony trabeculae were not unusual.

As regards the ligaments and tendons, the connective tissue bands were disrupted and replaced by nodular masses of acellular acidophilic material. Within the nodules were sinusoid-like vessels showing widely patent lumens filled with red blood cells and intact endothelial linings. In the uninvolved portions of connective tissue the vessels showed no change.

In the left eye the central artery of the retina showed an extensive deposit of amyloid, but its lumen was amply patent. There was no change in the retina. On the surface of the sclera the posterior ciliary vessels also showed severe amyloid involvement with virtual occlusion of the lumens.

Histologic sections of tracheobronchial lymph node, gallbladder, aorta, kidney, breast, uterus, pituitary, brain and meninges presented no significant change and no amyloid disease of the vessels.

Microscopic study of the pancreas, adrenal, esophagus, mesoappendix, thyroid and parathyroids showed amyloid infiltration of an occasional small artery. Otherwise there was nothing unusual.

The material in the various areas of deposit gave consistently positive reactions for amyloid. In the smaller nodules the stains were homogeneous, but the larger masses in the region of the joints showed coarse clumps of amyloid surrounded by lighter staining, almost nonspecific hyaline material. Some nodules presented a whorl-like appearance, as if the material had been deposited in irregularly concentric layers. Foreign body giant cell reaction at the periphery was frequent. The vessels within the nodules consisted of endothelial vascular slits completely surrounded by amyloid. There was no cellular infiltration. Special stains showed only a slight deposit of fat in the form of small droplets.

Amyloid involvement of blood vessels occurred chiefly in the small arteries. Usually the entire wall of the vessel was transformed to a homogeneous hyaline tissue without significant reduction of the lumen.

The final pathologic diagnosis was: primary systemic amyloidosis, with involvement of the skeletal muscles, lips, tongue, tendons, joints, bones, heart, and valves, intestinal tract and vagina; pathologic fracture of the left femur; cardiac hypertrophy and dilatation; chronic passive hyperemia of the lungs, liver and spleen; pulmonary arteriosclerosis, and bronchopneumonia.

COMMENT

Amyloid disease may be subdivided into four groups as follows: (1) secondary amyloidosis, (2) primary amyloidosis, (3) amyloidosis associated with multiple myeloma and (4) tumor-forming amyloidosis. Of these, secondary amyloidosis is relatively common and usually follows long-standing disease, such as tuberculosis or chronic suppuration. The liver, spleen, kidneys and adrenals are particularly involved, and the amyloid is deposited in a subendothelial position in the walls of capillaries and arterioles. In this form the amyloid gives typical staining reactions. Primary amyloidosis is characterized by absence of known etiologic factors. The amyloid is found in mesodermal tissue, smooth and skeletal muscle, the cardiovascular system and the gastrointestinal tract, while the organs usually affected in secondary amyloidosis, such as the liver and spleen, are uninvolved. The staining reactions of the amyloid are variable, and the substance tends to be deposited in nodular

as is known all of the patients were white. The duration of the disease from the onset of symptoms to death averaged approximately two and one-half years. In Lubarsch's first case the duration was eight years, and in our case it was fourteen years, which is the longest on record. In the majority of the patients the condition grew progressively worse, with a fatal termination due to intercurrent infection.

The clinical manifestations of primary systemic amyloidosis are fairly uniform. One of the most constant findings is enlargement of the tongue due to amyloid infiltration. While pain may be absent, the swelling and immobility of the organ result in dysphonia and dysphagia. In a number of cases the macroglossia has been mistaken for carcinoma. In extreme instances the patient is unable to close the mouth.¹⁰ The enlargement of the tongue is occasionally accompanied by swelling of the neck and face due to amyloid involvement of the skin, subcutaneous tissue or muscles. This may produce a fixed staring expression resembling that of a patient with paralysis agitans.⁸¹ A common symptom is progressive weakness and fatigue due to involvement of skeletal muscles. The clinical picture then simulates myotonia, although the muscles are enlarged and firm. In the case reported by DeNavasquez and Treble,^{3b} however, muscular weakness was caused by amyloid infiltration of the nerves. Disturbances in gait and limitation of motion are frequent and due to involvement of muscles, tendons and joints. In 5 cases there were complaints of pain in the back, extremities or region of the joints. Pain in the finger tips,¹¹ and hardening of the finger pads^{8s} may occur.

The skin was involved in 8 of the 24 cases. Königstein⁵ described an extensive eruption of sharply defined firm opalescent papules about the eyes and mouth and on the neck, trunk, extensor aspects of the extremities and fingers. Similar lesions of nodular type were also noted by Mollow and Lebell.⁸¹ In the patient observed by Michelson and Lynch⁸⁰ an eruption of the eyelids developed in the form of firm translucent waxy papules. In 2 cases reported by Lubarsch,^{3a} as well as in those reported by Gerstel^{8k} and Gaupp,⁴ the cutaneous lesions were of sclerodermic type. Gottron^{8j} described papular lesions of the skin of the thorax and abdomen in addition to a plaquelike scleroderma.

Infiltration of the intestine leads most frequently to constipation (8 cases). Other symptoms include diarrhea, abdominal pain, vomiting and meteorism. Intestinal hemorrhage occurred in 3 cases,¹² in 2 of which it was either the immediate cause of death or a contributing factor. In Steinhaus' ^{8b} case, in which the condition simulated carcinoma of the

10. Gottron.^{8j} Gerstel.^{8k}

11. Ritter.^{8c} Mollow and Lebell.⁸¹

12. Steinhaus.^{8b} Gerstel.^{8k} Michelson and Lynch.⁸⁰

pylorus, the bleeding was attributed to amyloid disease of the vessels. In the case reported by Michelson and Lynch⁸⁰ death was due to intestinal hemorrhage and ileus. The third case described by Lubarsch^{3a} concerned a 45 year old man whose chief complaint was hematemesis. There was a one year history of pain following meals, with anorexia and constipation. At autopsy there was amyloid disease of the stomach with a number of small pyloric ulcers showing a moderate amount of amyloid. Gottron⁸¹ obtained roentgen evidence of amyloid involvement of the stomach in the form of thickening of the mucosa and diminished peristalsis. Gerstel's^{8k} patient had severe diarrhea with melena and then intestinal obstruction. In the case observed by Mollow and Lebell⁸¹ there were persistent abdominal pain and constipation due to partial obstruction of the sigmoid colon.

Amyloid involvement of the heart occasionally leads to cardiac insufficiency. However, symptoms referable to cardiac failure may be difficult to evaluate. For example, dyspnea may be caused by involvement of the trachea, lungs or mediastinum or by the secondary anemia which is occasionally present. In the reports of 2 cases, however, the authors¹³ stated definitely that the extensive replacement of the cardiac muscle by amyloid contributed to the clinical picture of failure. Although the small arteries are often diffusely involved, the blood pressure is either normal or slightly low.⁸ⁿ Intermittent claudication was present in the case observed by Weber and his colleagues.^{8s}

Purpura is a frequent symptom and is presumably due to amyloid infiltration of blood vessels. The hemorrhages are most common in the skin but also occur in the tongue and mucous membranes. There may be a tendency to bruise readily.¹⁴ Hematemesis^{3a} and melena¹² have been described, and in the case of Picchini and Fabris^{8g} there were hemorrhages in the eyegrounds. Hematuria was present in 2 cases.¹⁵ Extensive cutaneous hemorrhages occurred in Lubarsch's^{3a} second case, in which a diagnosis of thrombocytopenic purpura was made. However, since the platelet count was 189,000 and the bleeding time three and one-half minutes, this diagnosis appears doubtful, and the bleeding may have been secondary to amyloid disease.

Other symptoms and signs include amenorrhea due to amyloid infiltration of the uterus^{8r} and dyspnea caused by extensive laryngeal involvement with obstruction.^{8g} Difficulty in micturition, impotence and pupillary changes in the patient observed by DeNavasquez and Treble^{3b} were ascribed to infiltration of the autonomic nervous system.

The distribution of the amyloid at autopsy was often very extensive. The most frequently affected organs were the tongue, heart, stomach,

13. Perla and Gross.^{2a} DeNavasquez and Treble.^{3b}

14. Michelson and Lynch.⁸⁰ DeNavasquez and Treble.^{3b}

15. Picchini and Fabris.^{8g} DeNavasquez and Treble.^{3b}

intestine and skeletal muscles. In 17 cases there was involvement of component organs of the cardiorespiratory, gastrointestinal and skeletal systems. The tongue was the seat of amyloid deposits in 20 of the 24 cases. Involvement of the heart occurred in 19 cases but in 2 of these was confined to the subepicardium.¹⁶ The weights of the affected hearts were usually within normal limits or slightly increased. However, the hearts of 2 patients weighed 545 and 750 Gm. respectively.¹⁷ Infiltration of the valves of the heart occurred in the instance reported by Wild,^{8a} as well as in the present case. There was amyloid in the gastrointestinal tract in 16 cases and in the skin in 8 cases. Involvement of the lungs, diaphragm, lymph nodes and serosal surfaces such as the peritoneum, pericardium and pleura was not uncommon. The involvement of lymph nodes may be universal¹⁸ or localized.¹⁹ Infiltration of skeletal muscles occurred in 10 cases, of tendons and joints in 4 and of bone in 2. Other sites of deposit include the lips, interior of the mouth, pharynx, esophagus, larynx, trachea, bladder, anus and practically the entire genital tract of the male and of the female. Involvement of the dura mater³ and of the vessels of the choroid plexus²⁰ and pia mater⁸¹ has been reported, but there is no instance of deposit in the brain. DeNavasquez and Treble^{3b} described infiltration of the posterior roots and sympathetic ganglions from the cervical, thoracic and lumbar regions and of peripheral nerves such as the ulnar, sciatic and peroneal. In the ganglions amyloid was found in nodular form between the ganglion cells and axis-cylinders. In many of the cases there was widespread involvement of small blood vessels, especially arteries, which in some instances included vessels in the liver, spleen and kidneys.

Primary systemic amyloidosis is a rare disease and consequently difficult to diagnose clinically unless its characteristics, distribution and fairly uniform group of symptoms and signs are kept in mind. It may simulate carcinoma of the tongue, scleroderma, myotonia, arthritis or any combination of these. In the case described by DeNavasquez and Treble^{3b} the association of cardiac failure, peripheral neuritis and diarrhea suggested beriberi. Involvement of the lungs may be mistaken for carcinoma.²¹ In 6 of the 23 cases collected the diagnosis was established by means of biopsy of tissues from various sites including the skin,²² tongue²³ buccal mucosa,⁸⁰ skeletal muscle²⁴ vagina^{8r} and finger tip.^{8s}

16. Gerstel.^{8k} Strauss.⁸ⁿ

17. Strauss.⁸ⁿ DeNavasquez and Treble.^{3b}

18. Lubarsch.^{3a} Strauss.⁸ⁿ

19. von Bonsdorff.^{8m} Gaupp.⁴

20. Mollow and Lebell.⁸¹ DeNavasquez and Treble.^{3b}

21. Strauss.⁸ⁿ Perla and Gross.^{2a}

22. Königstein.⁵ Gottron.^{8j} Reimann and others.^{8r}

23. von Bonsdorff.^{8m} Michelson and Lynch.⁸⁰ Reimann and others.^{8r} Weber and others.^{8s}

24. Gottron.^{8j} Weber and others.^{8s}

With respect to biopsies it should be remembered that the specific stains for amyloid may be atypical in color or faint in intensity; they may fade rapidly; in some instances they are entirely negative. In several cases the amyloid gave positive reactions with methyl violet but was weak or negative to iodine and sulfuric acid and to congo red.²⁵ In Gottron's²⁴ case, however, the congo red stains were positive and the methyl violet negative. The reactions may be typical in some areas of deposit and negative in other areas.²⁶ In some of the cases, including our own, the staining reactions were entirely typical.²⁷ Aid in diagnosis may be obtained by the use of the intravenous congo red test. Both positive²⁸ and negative²⁹ results have been reported with this method in cases of primary systemic amyloidosis. In respect to diagnosis, Lipstein's³⁰ recent evaluation of the congo red test for amyloidosis is of interest. In a series of 125 tuberculous patients he correlated the percentage of dye absorbed clinically with the presence or absence of amyloid at autopsy and concluded that the tests could be interpreted as confirmatory evidence of amyloid disease only when the percentage of dye absorbed was 90 or higher.

That multiple myeloma is frequently accompanied by amyloid disease is well known. This association is obviously too frequent to be merely coincidental. Magnus-Levy³¹ expressed the belief that the Bence Jones protein in cases of myeloma is chemically related to amyloid. Since the amyloid in cases of myeloma has the distribution and character of that in the primary form of the disease, the possibility of myeloma must be considered both clinically and at autopsy in every instance of amyloid disease of unknown cause. This is particularly true of amyloid involvement of joints and bone. In Glaus's³² case apparently primary systemic amyloid disease involving the tongue, heart, gastrointestinal tract, skin, skeletal muscles, lungs and bone marrow was shown at autopsy to be associated with multiple myeloma. Among the cases of primary systemic amyloidosis tabulated there is presumably no instance of an association of amyloidosis with myeloma. A number of authors have emphasized that they found no evidence of such a tumor. Myeloma was ruled out in our case. In the patient observed by Michelson and Lynch³⁰ the roentgenograms showed demineralization of bone suggesting disuse atrophy and were not diagnostic of myeloma. However, because of

25. Mollow and Lebell,⁸¹ Reimann and others.^{8r} Weber and others.^{8s}

26. Lubarsch,^{3a} Steinhaus.^{8b}

27. Königstein,⁵ Warren,^{8b} Gaupp,⁴ DeNavasquez and Treble.^{8b}

28. Gottron.²⁴ Michelson and Lynch.³⁰

29. von Bonsdorff.^{8m} Reimann and others.^{8r}

30. Lipstein, S.: *Am. J. M. Sc.* **195**:205, 1938.

31. Magnus-Levy, A.: *Ztschr. f. klin. Med.* **126**:62, 1934.

32. Glaus, A.: *Virchows Arch. f. path. Anat.* **223**:301, 1917.

Bence Jones proteinuria, the possibility of myeloma cannot be definitely excluded in their case without autopsy. As far as is known, Bence Jones proteinuria was not present in any other case, although examinations for this substance have apparently been performed in only a few instances.³³

In the present case of primary systemic amyloidosis there was widespread involvement of joints and secondarily of bone. The patient came under medical observation because of a pathologic fracture. The fourteen year duration of the disease is noteworthy. During the terminal two and one-half year period of clinical observation the patient remained in fair general health. In the following respects the condition in the present case is fairly typical of primary systemic amyloid disease: 1. There was no preceding or concurrent disease. 2. The involvement was limited to mesodermal structures, such as smooth and skeletal muscle and joints. 3. The amyloid was deposited in nodular form as illustrated in the intestinal and cardiac lesions. The amyloid gave consistently positive staining reactions with the congo red, methyl green, and iodine and sulfuric acid methods. Moreover, the results were positive with fresh tissue, with tissue fixed in solution of formaldehyde and with paraffin-embedded tissue. Special stains showed an insignificant amount of fat in the amyloid. This has been the general experience in other cases, although the presence of considerable amounts of fat has been reported.³⁴ The intravital congo red test was negative.

The extensive involvement of joints and of bones is of especial interest. Masses of amyloid replaced the articular capsules and ligaments of the joints and extended into the joint cavities and intracapsular portions of bone. This together with the involvement of tendons resulted in stiffness and partial ankylosis of the joints, with limitation of motion. The invasion of the left femur led to destruction of the entire neck of the bone and pathologic fracture. Although this fracture was complete and resulted in separation of the head of the femur, the mass of amyloid replacing the bone was so extensive and rigid that the patient was later able to resume slight bearing of weight.

Involvement of tendons and joints in primary systemic amyloidosis has been reported previously by Beneke,^{8d} Lubarsch^{3a} and von Bonsdorff.^{8m} In the cases of Gottron^{8j} and Michelson and Lynch^{8o} there were arthritic symptoms which suggested joint involvement, but no autopsies were performed. In a case of Beneke's^{8d} of primary local amyloid disease of the heart there was, in addition, diffuse involvement by amyloid of tendons and of the ligaments and capsules of the joints. In the first of Lubarsch's^{3a} 3 cases there were amyloid deposits in the

33. von Bonsdorff.^{8m} Strauss.⁸ⁿ Reimann and others.^{8r}

34. Lubarsch.^{3a} Gottron.^{8j} Mollow and Lebell.^{8l}

capsules of the knee and hip joints and in the adjacent tendons. Von Bonsdorff's³⁵ case is similar to our own. The articular capsules of the shoulder and elbow joints were replaced by amyloid, which invaded the joint spaces and by direct pressure eroded the head and lesser tubercle of the humerus and the olecranon of the ulna. This destruction of bone was demonstrated in roentgenograms during life and at autopsy. Amyloid tumors of the joints have been reported in association with multiple myeloma.³⁵

Primary amyloid disease of bone is rare. In generalized secondary amyloidosis bone is usually uninvolved, although there may be infiltration of the small vessels of the marrow. More common are secondary deposits of amyloid in bone or bone marrow the seat of chronic disease or within sarcomas³⁶ or blastomas of bone, especially multiple myeloma.³⁷ The present case and that of von Bonsdorff³⁵ are the only instances of bone involvement in primary systemic amyloidosis. Solitary primary amyloid tumors of bone involving the sixth and ninth right ribs have been recorded by Edens^{2c} and Hedrén,³⁸ respectively. In Edens' case there was also involvement of the liver, spleen, kidneys, stomach and intestine. Mandl³⁹ described a case of isolated primary amyloid tumor of the third thoracic vertebra with collapse of the bone. Pressure on the spinal cord produced transverse myelitis. Gerber^{2c} recently reported a case of atypical primary amyloid disease involving the liver, spleen and kidneys and diffuse amyloidosis of the bone marrow associated with collapse of the ninth thoracic and first lumbar vertebrae. In all these cases the amyloid was deposited in the reticulum and vessels of the marrow, and the destruction of bone was probably caused secondarily by the pressure of the infiltrating amyloid. In Mandl's³⁹ case, however, ischemia may have been a factor since the amyloid involvement of the small arteries resulted in virtual occlusion of their lumens. In none of the cases was there reactive formation of new bone.

Involvement of the cardiac valves in primary systemic amyloidosis is unusual, having been described previously only by Wild.^{3a} In his case the only information available is that the valve leaflets were

35. Buch, H.: Ein Fall von multipler primärer Sarcomatose des Knochenmarkes, und eine eigenthümliche Affection der vier grossen Gelenke, Med. Dissert., Halle, Lipke, 1873; cited by Magnus-Levy.³¹ Zeehuisen, H.: Nederl. tijdschr. v. geneesk. 29:829, 1893; cited by Magnus-Levy.³¹ Hueter, C.: Beitr. z. path. Anat. u. z. allg. Path. 49:101, 1910. Paige, B. H.: Am. J. Path. 7:691, 1931.

36. Hildebrand, O.: Virchows Arch. f. path. Anat. 140:249, 1895.

37. Rosenblum, A. H., and Kirshbaum, J. D.: J. A. M. A. 106:988, 1936. Freund, E.: Frankfurt. Ztschr. f. Path. 40:400, 1930. Magnus-Levy.³¹

38. Hedrén, G.: Ztschr. f. klin. Med. 63:212, 1907.

39. Mandl, J.: Virchows Arch. f. path. Anat. 253:639, 1924.

thickened as a result of extensive amyloid disease. In Israel's⁴⁰ case amyloidosis involved the mitral, tricuspid and pulmonic valves as well as the heart, mouth, larynx, skin and mediastinum, but the deposition of amyloid may have been due to cirrhosis of the liver. Involvement of the valves in isolated primary amyloid disease of the heart is usually slight,⁴¹ although in Koller's⁴² case the mitral and tricuspid valves were the seat of extensive deposits. In describing the experimental amyloidosis produced in mice by injections of sodium caseinate Jaffé⁴³ mentioned lesions of the cardiac valves. The involvement was found mainly in the mitral leaflets, where the amyloid was deposited as a compact layer near the auricular surface. In the present case the severe amyloid involvement of the valves resulted in deformity and circulatory changes. The aortic valve presented thick, shortened cusps, so completely immobile as to preclude their ability to flap or to approximate in diastole. The mitral leaflets were likewise thickened, stiff and immobile. Both valves presented rigid stenotic orifices. The severe passive hyperemia of the lungs was comparable to that seen with chronic rheumatic mitral stenosis. There were hypertrophy and dilatation of the right ventricle, passive hyperemia of the liver and other viscera and hypertrophy of the left ventricle, which was attributed to the aortic valvular disease. The amount of amyloid deposited in the ventricles was not sufficiently great to contribute to their hypertrophy. Examination of the heart failed to reveal evidence of healed rheumatic disease. Numerous microscopic sections of the free portions of the cardiac valves showed considerable deposit of amyloid, but none was found in the blood vessels. Elsewhere in the body, even in such relatively avascular structures as tendons and the ligaments of joints, the small blood vessels in the neighborhood of amyloid nodules, especially arteries, almost invariably showed amyloid infiltration. The extensive involvement of the leaflets may perhaps be explained by direct extension of amyloid from the ring of the valve.

The origin of amyloid in both the primary and the secondary form of amyloidosis is still obscure. Little is known of the nature of amyloid other than what is known of its protein content, homogeneous character and staining reactions. The variable staining of the material in the primary disease as contrasted with the uniform staining in the secondary form indicates that the two types are not identical. The material

40. Israel, I.: Ein Fall von lokalem Amyloid, Med. Dissert., Tübingen, Bochum-Langendreer, 1933.

41. (a) Budd, J. W.: Am. J. Path. **10**:299, 1934. (b) Larsen, R. M.: *ibid.* **6**:147, 1930.

42. Koller, F.: Schweiz. med. Wchnschr. **13**:522, 1932.

43. Jaffé, R. H.: Arch. Path. **1**:25, 1926.

recognized histologically as amyloid probably has a variable chemical composition and comprises a group of closely related substances.

The pathogenesis of primary systemic amyloidosis is unknown. Whether the amyloid is the product of infiltration or of degeneration is not clear. The regularity with which the substance is restricted to muscle and connective tissue is significant. Warren^{5h} pointed out that since amyloid may be formed in connective tissue at a considerable distance from blood vessels, the substance is the product of abnormal fibroblastic activity. Reimann, Koucky and Eklund^{4r} suggested a generalized metabolic perversion of tissue of mesodermal origin. Larsen,^{41b} however, in a study of primary myocardial amyloidosis concluded that amyloid is always deposited primarily about venocapillary endothelium. By means of serial sections he established the continuity of nodules of amyloid in isolated areas with deposits around the endothelium of vessels.

Letterer⁴⁴ expressed the belief that an etiologic relationship exists between amyloidosis and hyperglobulinemia. There is experimental evidence to support this idea. Reimann and Eklund⁴⁵ found that increase in blood globulin regularly accompanied the amyloidosis which they produced in rabbits by injections of sodium caseinate. Dick and Leiter⁴⁶ also noted hyperglobulinemia in association with the amyloidosis produced in rabbits by the use of various strains of streptococci. Amyloid disease occurs frequently in horses used to produce various antiserums,⁴⁷ and such animals show an increase, often marked, in serum globulin.⁴⁸ Clinically, only a few determinations of blood protein have been made in cases of primary systemic amyloidosis, and these were within normal limits.⁴⁹ Hyperglobulinemia is rare with secondary amyloidosis. It has been reported in cases of multiple myeloma, in which the increase in plasma protein was almost entirely in the euglobulin fraction.⁵⁰ The experimental findings suggest that amyloid may represent a reaction between some component of the serum globulin and certain fixed tissue elements which in the case of primary amyloidosis are present in mesoblastic structures or in the walls of

44. Letterer, E.: *Beitr. z. path. Anat. u. z. allg. Path.* **75**:486, 1926.

45. Reimann, H. A., and Eklund, C. M.: *Am. J. M. Sc.* **190**:88, 1935.

46. Dick, G. F., and Leiter, L.: *Tr. A. Am. Physicians* **52**:246, 1937.

47. Doerken, E.: *Virchows Arch. f. path. Anat.* **286**:487, 1932. Arndt, H. J., and Doerken, E.: *Arch. f. wissenschaft. u. prakt. Tierh.* **63**:1, 1931.

48. Reitstötter, J.: *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **30**:468, 1920.

49. Reimann and others.^{8r} DeNavasquez and Treble.^{3b}

50. Perlzweig, W. A.; Delrue, G., and Geschickter, C.: *J. A. M. A.* **90**:755, 1928. Shirer, J. W.; Duncan, W., and Haden, R. L.: *Arch. Int. Med.* **50**:829, 1932.

capillaries or arterioles. Such a reaction may possibly be of antigen-antibody nature and may have an allergic basis.

SUMMARY

A case of primary systemic amyloidosis is reported. The duration of the disease was fourteen years. There was extensive involvement of the joints and of bones, with pathologic fracture of the left femur, and there was extensive amyloid infiltration of the valves of the heart.

Primary systemic amyloidosis, although rare, has become a recognized entity. There are now in the literature reports of 23 cases. The disease is apparently primary and is characterized by a general distribution of amyloid in mesodermal structures of the body, especially in smooth and skeletal muscle. The most frequently affected organs are the tongue, heart, stomach, intestine and skeletal muscles. The liver, spleen and kidney, usually affected in the secondary form of amyloidosis, are rarely involved.

The genesis of the disease is obscure. Whether the amyloid is the result of infiltration or of degeneration of mesodermal tissue is not clear. The atypical staining of the amyloid in the primary form as contrasted with the uniform staining in secondary amyloidosis suggests that the two types are not identical. The material recognized histologically as amyloid probably comprises a group of substances closely related in chemical structure.

SENSITIZATION, ANTIBODY FORMATION AND LESIONS PRODUCED BY TUBERCLE BACILLI IN THE ALBINO RAT

EDWARD HEHRE, M.D.

AND

JULES FREUND, M.D.

NEW YORK

Experimental tuberculosis has been studied mainly in the guinea pig and rabbit. The former species is used because it contracts tuberculosis with little resistance, and the latter, because it is highly susceptible to infection by the bovine type of tubercle bacilli. It seems desirable to approach some questions of pathogenesis and immunity through studies in a highly resistant species, the albino rat. Tuberculosis in the rat may offer opportunities to correlate some aspects of the disease under conditions not observed in the highly susceptible species of animals.

In general, the rat stands apart from man and many lower animals in regard to phenomena of hypersensitiveness and antibody formation. Anaphylaxis is demonstrable with difficulty in the rat (Longcope¹; Parker and Parker²) and the Arthus phenomenon cannot be induced (Longcope¹; Opie³). Neither has the tuberculin skin reaction or the Koch phenomenon been elicited in this species (Boquet and Nègre;⁴ Gloyne and Page;⁵ Ornstein and Steinbach;⁶ Boquet, Nègre and Valtis;⁷ M. I. Smith;⁸ W. Jadassohn⁹).

Some workers have attempted to correlate the allergic reactivity of the skin of the guinea pig and of the rabbit with tubercle formation and caseation. The lack of skin reactivity in the rat when studied in conjunction with the pathogenesis of tuberculosis may throw additional

From the Department of Pathology, Cornell University Medical College.

1. Longcope, W.: *J. Exper. Med.* **36**:627, 1922.
2. Parker, J. T., and Parker, F., Jr.: *J. M. Research* **44**:263, 1924.
3. Opie, E. L.: *J. Immunol.* **9**:231, 1924.
4. Boquet, A., and Nègre, L.: *Ann. Inst. Pasteur* **35**:142, 1921.
5. Gloyne, S. R., and Page, D. S.: *J. Path. & Bact.* **26**:224, 1923.
6. Ornstein, G. G., and Steinbach, M. M.: *Am. Rev. Tuberc.* **12**:77, 1925.
7. Boquet, A.; Nègre, L., and Valtis, J.: *Compt. rend. Soc. de biol.* **97**:1665, 1927.
8. Smith, M. I.: *Pub. Health Rep.* **43**:2817, 1928.
9. Jadassohn, W.: *Arch. f. Dermat. u. Syph.* **167**:169, 1933.

light on the possible relationship of allergy to some aspect of the disease, for instance, caseation.

Many attempts have been made to produce "tuberculin death" in the rat. Most of them have failed (Boquet and Nègre;⁴ Gloyne and Page;⁵ Ornstein and Steinbach;⁶ Boquet, Nègre and Valtis;⁷ Schütze and Zilva¹⁰). Smith and Hendrick¹¹ found that the intraperitoneal injection of 0.5 cc. of tuberculin killed few tuberculous rats when they were kept on a normal diet but killed most of them when they were fed on a diet deficient in vitamin A. More recently, M. I. Smith⁸ studied the effect of an intravenous injection of a filtrate (protein) from cultures of tubercle bacilli on tuberculous rats. He found that 10 mg. of protein killed 1 of 12, 50 mg. 6 of 10 and 100 mg. all of 10 tuberculous rats. All normal rats were resistant to 200 mg., but 3 of 5 died after an injection of 300 mg. Lack of vitamin A made both normal and tuberculous rats more susceptible to the lethal effect of tuberculo-protein. Rats submitted to intraperitoneal injection of heat-killed tubercle bacilli were not sensitive to tuberculin.

The rat seems to be an unusual species in regard to antibody formation. The production of antibodies against either toxins or foreign proteins is scant (Coca and others;¹² Longcope;¹ Parker and Parker²). Ornstein and Steinbach⁶ reported that rats infected with tubercle bacilli do not produce antibodies.

The first systematic investigation of the pathogenesis of tuberculosis in the rat was made by the British Royal Commission on Tuberculosis.¹³ They concluded that the rat is highly resistant to infection with tubercle bacilli and that infection in the rat differs conspicuously from that in man and susceptible lower animals. The commission found that when the rat is infected subcutaneously with tubercle bacilli or is fed tubercle bacilli it fails to present generalized tuberculosis. After intraperitoneal or intravenous injection of very large doses of human or bovine tubercle bacilli rats may die with the Yersin¹⁴ type of tuberculosis, which is characterized by abundant multiplication of organisms in the tissues in the absence of tubercles. Gloyne and Page,⁵ using rats, injected 1,000,000,000 human tubercle bacilli into the subcutaneous tissue, peritoneal cavity or testis and observed the results for forty-five days.

10. Schütze, H., and Zilva, S. S.: *J. Hyg.* **26**:204, 1927.

11. Smith, M. I., and Hendrick, E. G.: *J. Lab. & Clin. Med.* **11**:712, 1926.

12. Coca, A.; Russell, E. F., and Baughmann, W. H.: *J. Immunol.* **6**:387, 1921.

13. Report of the Royal Commission on Tuberculosis (1907-1911), cited by Cobbett, L.: *The Causes of Tuberculosis*, Cambridge, University Press, 1917, p. 443; cited by Gloyne and Page;⁵ cited by Griffith, A. S.: *Experimental Tuberculosis*, in *A System of Bacteriology*, Privy Council, Medical Research Council, London, His Majesty's Stationery Office, 1930, vol. 5, p. 169.

14. Yersin, M. A.: *Ann. Inst. Pasteur* **2**:245, 1888.

Macroscopic tubercles were found in the lungs of only a few animals. Microscopic lesions were seen in the lungs, spleen, liver, lymph nodes and bone marrow. These comprised poorly stained collections of phagocytes with tubercle bacilli in and around them. Lange¹⁵ found that rats which received approximately 1,500,000 bovine tubercle bacilli into the subcutaneous tissue acquired histologic tubercles at the sites of injection and in the draining lymph nodes. The tubercles and tubercle bacilli gradually disappeared. Ornstein and Steinbach⁶ gave rats an intraperitoneal injection of 0.33 mg. of a human strain of low virulence (H37). The animals were killed from three to ninety-three days after infection. Macroscopic or microscopic tubercles were not found, but tubercle bacilli were present in smears and sections of many organs. The authors were able to infect guinea pigs with the rat tissues. More recently Steinbach¹⁶ has confirmed and extended these observations. Rats that received 1 mg. of human (H37) or bovine (B1) tubercle bacilli intraperitoneally had no macroscopic or microscopic tuberculous lesions from twenty-five to two hundred and six days after inoculation, although the bacilli were found in smears and histologic sections of the viscera. Rats subjected to adrenalectomy or thyroparathyroidectomy and similarly infected with bovine tubercle bacilli of the B1 strain had macroscopic and microscopic tubercles of the spleen, liver, omentum, retroperitoneal lymph nodes and diaphragm within two months after infection. Caseation was often observed.

Smith and Hendrick¹¹ studied tuberculous lesions in rats given an intraperitoneal injection of 5 mg. of a human strain of tubercle bacilli of low virulence (H37). They observed large multinucleated cells with eosinophilic cytoplasm and occasional giant cells in the omentum, spleen, liver and lymph nodes, as well as aggregates composed of epithelioid cells, large multinucleated epithelioid cells, occasional giant cells and lymphocytes in the lungs. Caseation was never observed, but intracellular lipid globules were found in the epithelioid cells in the lungs. Large numbers of bacilli were found in the lungs of rats dying one year after infection. The other organs contained few tubercle bacilli. Long and Vorwald¹⁷ and Vorwald¹⁸ examined histologic sections of lungs of rats six months after an intravenous injection of approximately 0.01 mg. of human tubercle bacilli (H37). They found typical tubercles, containing tubercle bacilli, with no caseation.

The plan of the present work was to study the cutaneous sensitiveness, the systemic reaction to tuberculin and the production of comple-

15. Lange, L. B.: *Am. Rev. Tuberc.* **7**:49, 1923; **11**:241, 1925; **15**:629, 1927.

16. Steinbach, M.: *Am. Rev. Tuberc.* **26**:52, 1932.

17. Long, E. R., and Vorwald, A. J.: *Nat. Tuberc. A. Tr.* **26**:205, 1930.

18. Vorwald, A. J.: *Am. Rev. Tuberc.* **27**:270, 1933.

ment-fixing antibodies in rats after an injection of heat-killed tubercle bacilli or after infection. The pathogenesis of tuberculosis was studied in normal and immunized animals.

MATERIAL AND METHODS

Mature male albino rats weighing from 130 to 170 Gm. were used. The diet was a mixture of hominy, sodium chloride, rolled oats, meat scraps, skimmed milk and wheat, which was fed four times a week; a mixture of lettuce and cauliflower or broccoli stems was fed three times a week; and dog biscuits^{18a} were given twice a week. Rats have been maintained on this diet for several years and have not shown signs of avitaminosis.

The tubercle bacilli used were of the bovine type, strain Ravenel, isolated more than twenty years ago by Dr. M. Ravenel. This strain possesses high virulence, so that 0.00001 mg. injected intravenously kills rabbits with extensive pulmonary and renal tuberculosis in from three to six months. Rats were infected with 1 mg. and occasionally 10 mg. of this strain. Injections were made through a 29 gage hypodermic needle into the exposed left femoral vein.

A suspension of killed tubercle bacilli was employed both as an immunizing agent and as antigen for complement fixation. It was prepared in the following manner: Weighed amounts of bacillary growth on glycerin agar, approximately five weeks old, were ground and suspended in saline solution; 1 cc. of the suspension contained 10 mg. The suspension was heated in an Arnold steam box for half an hour at 100 C. and preserved with 0.35 per cent cresol, U. S. P.

Tuberculin prepared from cultures of the Ravenel strain was approximately one and a half times as potent as the international standard tuberculin. As a rule, 0.1 cc. of a 1 in 5 dilution was injected into the skin. Varying quantities of the same dilution were used for intraperitoneal injection.

Complement fixation was employed to determine the antibody titer of the blood. Fresh rat serums were inactivated by heating at 55 C. for thirty minutes. Approximately one fourth of the self-inhibiting dose of the antigen was used in the complement fixation. The complement, namely, guinea pig serum, was always freshly titrated, and 2½ units was used. For the test 0.25 cc. of each of the aforementioned ingredients was incubated in a water bath at 37 C. for one hour. Then 0.25 cc. of a suspension of washed sheep cells (in one twentieth of their concentration in whole blood) and 0.25 cc., or 2 units, of antisheep hemolysin was added. The whole system was then incubated at 37 C. until the standard serum indicated completion of the second phase. To insure the accuracy of the test, a standard serum was always included. This was obtained from a rabbit that had been immunized by a series of injections of tubercle bacilli and was preserved in the

18a. Purima Dog Chow contains:

Wheat germ	Dried meat
Barley	Molasses
Carotene (vegetable compound)	Corn grit
Malt	Cereal feed
Dried beef pulp	Cod liver oil
Dried skimmed milk	Iodized salt
Brewers' dried yeast	

Protein, 20 per cent; fat, 6 per cent; fiber, 6 per cent, and nitrogen-free extract, 46 per cent.

dried state in 0.5 cc. portions according to the method of Elser, Thomas and Steffen¹⁹ or that of Flosdorf and Mudd.²⁰ Each sample of dried serum was recovered as needed by the addition of 0.5 cc. of distilled water. That the method of drying yielded uniform samples was shown by testing 3 samples of dried serum simultaneously. The variation, if any, in the complement fixation tests over a period of months was slight, since the highest dilution of the standard serum that fixed complement was always the same.

The methods used in the immunization of rats with heat-killed tubercle bacilli were as follows: The animals of group A received a weekly intracutaneous injection of 0.1 mg. of heat-killed tubercle bacilli, respectively, for eight weeks. The rats in group B received, in all, seven intracutaneous injections of 0.2 mg. of heat-killed tubercle bacilli, given at four day intervals. The animals of group C were each given seven simultaneous subcutaneous injections of 0.2 mg. of heat-killed tubercle bacilli, followed by weekly subcutaneous injections of 0.2 mg. each

TABLE 1.—*Tuberculin Reactions in Normal and Tuberculous Rats*

Group	Tuberculin		Rat	Days After Infection	Skin Reaction	Systemic Reaction	Death from Reaction to Tuberculin
	Mg.	Route					
Normal	20	Intracutaneous	8, 9	...	0	0	0
	100	Subcutaneous	58, 59, 61, 63	...	0	0	0
	800	Intraperitoneal	51	0	0
	1,000	Intraperitoneal	52	0	0
Infected	20	Intracutaneous	16	13, 29	0	0	0
			11, 17	13, 29, 46	0	0	0
			20	13, 29, 46, 70	0	0	0
			11	70	0	—	In 2 days
			20	138	0	+	0
			23	145	0	+	In 2 days
			22*	145	0	+	Within 1 day
			51, 52, 54	164	0	+	0
			50	175	0	+	0
	20	Intraperitoneal	51, 52	31, 84	.	+	0
			57	34	..	+	Within 1 day
			54	84	.	+	0
			50	95	..	+	0
	100	Intraperitoneal	53	14	.	+	Within 1 day
			24, 25	96	..	+	Within 1 day

* This rat was infected with 10 mg. of tubercle bacilli, injected intravenously.

for four weeks. Some of these animals (constituting group D) received one or two intraperitoneal injections of 0.2 mg. of heat-killed bacilli within twenty-one days after the last subcutaneous injections.

EXPERIMENTAL OBSERVATIONS

Hypersensitiveness.—Ten tuberculous rats were tested by injecting intracutaneously into each 20 mg. of tuberculin at one or more intervals varying from thirteen to one hundred and seventy-five days after infection. In no instance was a positive skin reaction, i. e., redness and edema, obtained. In contrast to the lack of skin reaction, systemic reactions occurred in some of the rats so treated (table 1). The rats

19. Elser, W. J.; Thomas, R. A., and Steffen, G. I.: J. Immunol. **28**:433, 1935.

20. Flosdorf, E. W., and Mudd, S.: J. Immunol. **29**:389, 1935.

tested thirteen, twenty-nine and forty-six days after infection gave no evidence of hypersensitiveness, but 3 of 4 rats that received 20 mg. of tuberculin intradermally from seventy to one hundred and forty-five days after infection became ill and died within two days after the injection. Four rats that were tested with tuberculin from one hundred and sixty-four to one hundred and seventy-five days after infection became ill, as was indicated by irritability and ruffling of the body hair, but survived the injection. Twenty milligrams of tuberculin was injected into the peritoneal cavity of each of 5 tuberculous animals at one or more intervals varying from thirty-four to ninety-five days after infection. The symptoms of irritability and ruffling of hair followed each injection, but only a single rat died. In a final experiment 1 rat, infected fourteen days previously and 2 rats, infected ninety-six days previously, were given an intraperitoneal injection of 100 mg. of tuberculin. All died within twenty-four hours. These observations suggest that the

TABLE 2.—*Tuberculin Reactions in Rats Immunized with Heat-Killed Tubercle Bacilli*

Tuberculin		Immunization Group	Animals	Days After Immunization	Skin Reaction	Systemic Reaction	Death from Reaction to Tuberculin
Mg.	Route						
20	Intracutaneous	A	5	6	0	0	0
		B	5	4	0	0	0
		C	2	9	0	0	0
1,000	Intraperitoneal	D	1	29	..	+	0
		D	1	29	..	+	In 5 days

site of injection, i. e., the skin or the peritoneal cavity, did not influence the systemic effect of tuberculin. Table 1 also shows that neither cutaneous reaction to nor death from tuberculin occurred in normal rats. As large an amount as 1,000 mg. of tuberculin injected into the peritoneal cavity of a normal rat failed to cause noticeable general reaction.

The injection of heat-killed tubercle bacilli into the skin of normal rats was followed by the formation of nodules from 2 to 4 mm. across and less than 0.5 mm. high at the sites of injection. These nodules did not increase in size or ulcerate. This reaction to the antigen did not change when the injection was repeated. Rats repeatedly given injections of heat-killed tubercle bacilli did not show local reactions to 20 mg. of tuberculin injected into the skin (table 2), and systemic reactions did not follow these injections. On introduction of 1,000 mg. of tuberculin into the peritoneal cavity of each of 2 immunized rats, general malaise developed in both. One died after five days.

In rats first immunized with heat-killed tubercle bacilli and later infected with living tubercle bacilli skin reactions to tuberculin did not occur. However, systemic reactions and death were produced regu-

larly and promptly (table 3). A comparison of tables 1 and 3 suggests that the injection of heat-killed tubercle bacilli prior to infection made the rats more susceptible to the toxic action of tuberculin.

Antibody Formation.—Complement fixation tests were performed on 46 samples of serum from 31 normal rats. From some of the rats samples of blood were obtained on two occasions. Complement fixation was observed regularly with undiluted rat serums and frequently (47 per cent) with serums diluted 1 in 2.5. As a rule, when serums were diluted 1 in 5 fixation did not occur. There were 5 serums that fixed complement in such a dilution (11 per cent). None of the serums reacted in dilutions of 1 in 10, 1 in 20 or 1 in 40.

Complement fixation was tested with the serums of 12 rats infected with 1 mg. or 10 mg. of tubercle bacilli. With 2 rats, tests were made forty-nine, eighty-nine and one hundred and forty-five days after infec-

TABLE 3.—*Tuberculin Reactions in Tuberculous Rats That Had Been Immunized with Heat-Killed Tubercle Bacilli Prior to Infection*

Tuberculin		Rat	Immunization Group	Days After Infection	Skin Reaction	Systemic Reaction	Death from Reaction to Tuberculin
Mg.	Route						
20	Intraeantaneous	32	D	25	0	+	0
		33	D	31	0	+	0
		46	D	45	0	+	Within 1 day
		6	A	96	0	+	0
		5, 10, 14	A	96	0	+	Within 1 day
		6	A	171	0	+	Within 1 day
100	Intraperitoneal	31	D	18	..	+	Within 1 day
200	Intraperitoneal	27	D	18	..	+	Within 1 day

tion. The tests were carried out as a rule with serum dilutions from 1 in 5 to 1 in 40. Complement fixation was obtained with 4 serums in 1 in 5 dilution. A serum in this group failed to give complement fixation when retested on two later occasions. With serums diluted higher than 1 in 5, fixation was never observed.

While an increase in complement-fixing antibodies was not observed in rats that had been merely infected with tubercle bacilli, a significant rise in antibody titer was noted in those animals that had received injections of tuberculin in addition to infection (table 4). Of 7 serums from rats that had been infected from one to five months previously with 1 mg. of tubercle bacilli and subsequently given 20 mg. of tuberculin on one or more occasions, 5 fixed complement in a dilution of 1 in 10, and 2 showed fixation in a dilution of 1 in 20. One of these when retested several months later was negative in a dilution of 1 in 10. Two serums from rats that had received large intraperitoneal injections of tuberculin before infection, as well as subsequent small doses, showed the same titers of antibody as the serums of infected animals that had received only small doses of tuberculin. A rat that died following an

intracutaneous injection of 20 mg. of tuberculin gave serum post mortem that failed to fix complement in a dilution of 1 in 5.

The repeated injection of heat-killed tubercle bacilli into normal rats induced formation of antibodies regardless of the mode of immunization (table 5). A higher percentage of the animals immunized by the intracutaneous route (groups A and B) showed increase in antibodies

TABLE 4.—*Antibody Titers of Serums of Tuberculous Rats After Injections of Tuberculin*

Tuberculin		Rat	Days Between Infection and Injections of Tuberculin	Days Between Infection and Complement Fixation	Titer
Mg.	Route				
20	Intracutaneous	16	13, 29	31	10
		11*	13, 29, 46, 70	72	0
		20	13, 29, 46, 70, 135	157	10
20	Intraperitoneal	51, 52	34	48	10
		51, 54	34, 84	98	20
		52	34, 84	98	5
		56	45, 95	109	5

* The blood used was obtained after death from reaction to tuberculin.

TABLE 5.—*Antibody Titers of Serums of Rats Immunized with Heat-Killed Tubercle Bacilli*

Rat	Immunization Group	Days After Immunization	Titer
14	A	8	10
5			20
6			40
M9	B	6	0
M1, M5, M8			5
M3			20
27, 28, 31, 33, 34, 35, 36, 38, 40, 46	C	6	0
29, 39			10
32			40
35, 36	D	18	10
33, 38, 40			20
31, 39, 46			40
27, 32	D	30	80
40			20
27			40
33, 35, 36	D	36	20
27, 31			40
32			80

than of those given subcutaneous injections (group C). The highest antibody titers were found when rats had been given one or two intraperitoneal injections of 0.2 mg. of heat-killed tubercle bacilli subsequent to subcutaneous injections (group D). The serums of 5 of 10 rats so treated fixed complement in a dilution of 1 in 40, and 2 of these serums were positive in a dilution of 1 in 80.

The serums of 7 tuberculous rats that had received a series of intracutaneous injections of heat-killed tubercle bacilli before infection did not fix complement at appreciably higher dilutions than the serums of

nonimmunized tuberculous animals (table 6). Two rats given intracutaneous injections of 20 mg. of tuberculin ninety-six days after infection had high antibody titers.

Tuberculosis in the Rat.—Eight adult male albino rats were infected intravenously with 1 mg. of the Ravenel strain of bovine tubercle bacilli. Three died between twenty-six and eighty-seven days after infection. Two died six months after infection. One died ten months after, another one year after, while still another survived eighteen months. It is uncertain to what extent tuberculosis shortened the normal life expectancy of these animals, since we had no control series of noninfected animals. Many of the rats had extensive nontuberculous pulmonary lesions, i. e., bronchiectasis and abscesses, that evidently preceded tuberculous infection. The only gross lesions of tuberculosis induced in these rats were minute tubercles in the lungs and enlargement of the spleen. The great resistance of the rat to infection with

TABLE 6.—*Antibody Titers of Serums of Tuberculous Rats That Had Been Immunized with Heat-Killed Tubercle Bacilli Previous to Infection*

Immunization Group	Rat	Days After Infection	Titer
A	5, 14	75	0
	6	75	5
	14*	97	40
	6*	115	20
B	M9	29	0
	M8	35	5
	M1	42	5
	M5	49	0

* In this rat 20 mg. of tuberculin had been injected intracutaneously ninety-six days after infection.

tubercle bacilli is apparent; for, if rabbits are infected intravenously with 0.00001 mg. of the same strain, they succumb within from three to six months and at autopsy present massive tuberculosis of the lungs and kidneys (Opie and Freund²¹).

Since the pathogenesis of tuberculosis in rats has been inadequately described and is a subject of controversy, a description of the pathologic changes in the animals observed in this study is desirable. Post-mortem examinations were made of 26 rats that died or were killed from two hours to eighteen months after the intravenous injection of 1 mg. of bovine tubercle bacilli. Parts of the lungs, spleen, liver and kidneys were fixed in Zenker's fluid, embedded in paraffin and cut 6 microns in thickness. Duplicate sections were stained with hematoxylin and eosin and by the Ziehl-Neelsen method. The fate of the injected tubercle bacilli was followed by counting the number of acid-fast rods in 100 oil immersion fields (Zeiss, $\times 900$) of the sections stained by the Ziehl-Neelsen method.

21. Opie, E. L., and Freund, J.: J. Exper. Med. 66:761, 1937.

Lung: The lungs of all animals infected more than one month previously showed minute grayish white nodules, discrete and confluent, uniformly distributed over their pleural and cut surfaces (fig. 1 *A*). These gross tuberculous lesions were differentiated from the spontaneous abscesses of the lungs found in many rats by their small size, gray color and uniform distribution. Histologic examination revealed tuberculous lesions in various stages of development in all of the animals.

Two hours after infection, clumps of acid-fast bacilli were found in the alveolar capillaries in the subpleural region, and occasional bacilli were seen in single polymorphonuclear leukocytes or large mononuclear cells in the alveolar septums. From three to fifteen days after infection, tubercle bacilli were found in round collections of large mononuclear cells in the alveolar septums. After the second week, these simple tubercles, measuring 20 to 60 microns in diameter, changed little in size and were often intra-alveolar. The cells that formed them had the character of epithelioid cells and often contained acid-fast bacilli. In them, also, were granules that assumed a brown color but were not acid-fast by the Ziehl-Neelsen method. The cells had light staining oval or irregularly elongated nuclei, containing a few coarse chromatin granules, a prominent nucleolus and a heavy folded nuclear membrane. Their cytoplasm was abundant, lightly acidophilic and reticulated, and the cell boundaries were often indistinct. Single necrotic epithelioid cells were seen in many of the tubercles, but caseation was never observed. Occasional giant cells of the Langhans type, containing bacilli and granules, were found within the pulmonary alveoli of most animals after the second week.

The minute tubercles that we saw on the surfaces of the lungs of all rats after one month (fig. 1 *A*) were subpleural groups of epithelioid cells, in part within the septums and in part intra-alveolar; i. e., associated with a small tubercle there was a subpleural focus of tuberculous pneumonia (fig. 1 *B*).

Table 7 shows the number of acid-fast rods demonstrable in 100 oil immersion fields in sections of lung. Two hours after infection, approximately 200 bacilli per hundred fields were demonstrable. Fewer tubercle bacilli were found in 14 animals that were killed within seventy-two days after infection, although an animal that died twenty-six days after infection had a very large number of organisms in the lungs. After seventy-three days, the figures varied but were on the whole higher than in the earlier period. In 3 animals that died six, twelve and eighteen months after infection the number of tubercle bacilli in the lungs was high, i. e., several thousand per hundred oil immersion fields.

The lesions in animals that had tubercle bacilli in great numbers in their lungs were similar to those in the other rats. Caseation was

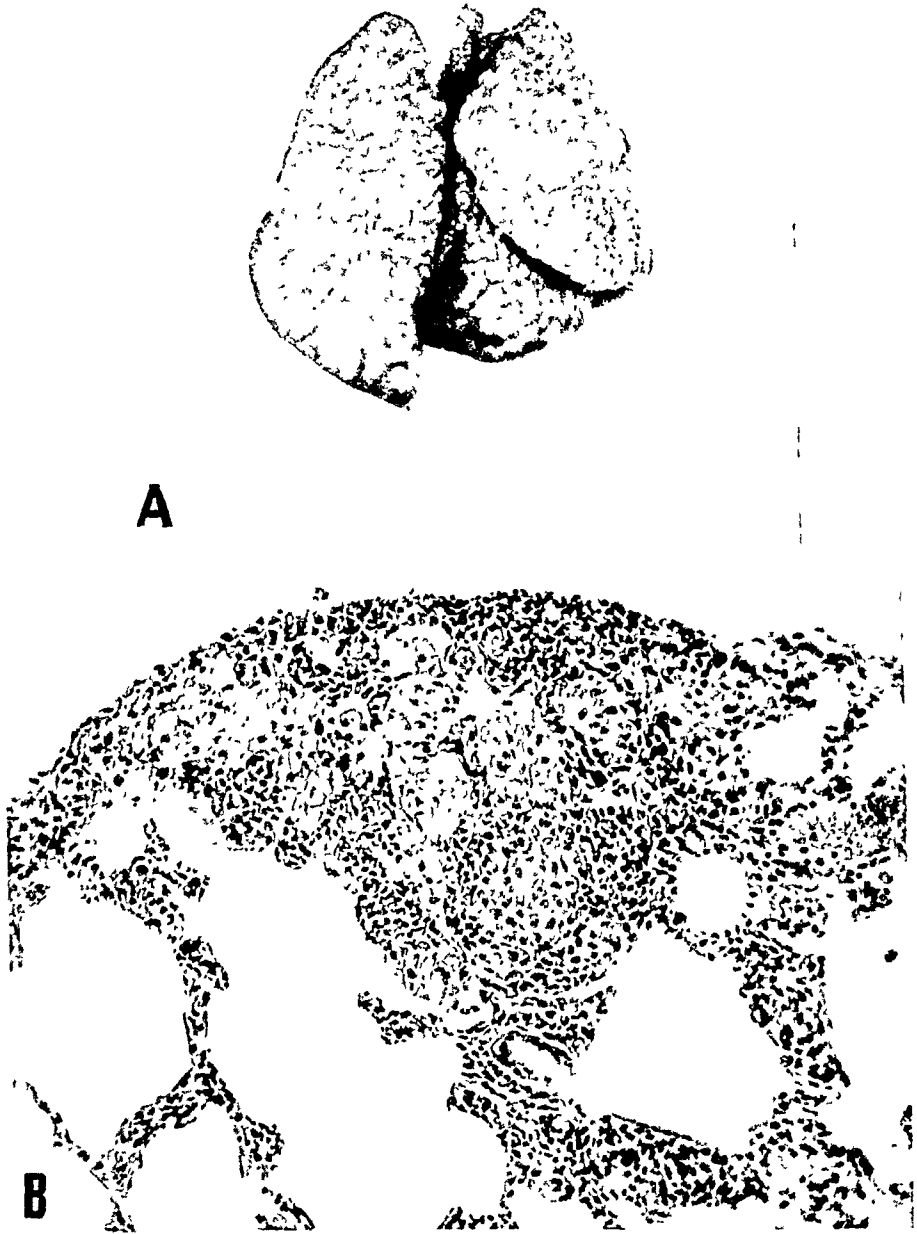


Fig. 1.—*A*, posterior aspect of the lungs of rat 51 which died ten months after an intravenous infection with 1 mg. of bovine tubercle bacilli. Note the discrete and confluent tubercles distributed over the surfaces of these lungs (natural size). *B*, section through a tubercle below the surface of the lung of rat M12, which was killed forty-nine days after infection; $\times 200$. The tubercle is a group of septal and intra-alveolar collections of epithelioid cells in which tubercle bacilli are found. Note the Langhans giant cell.

absent, and the number of epithelioid cells with necrosis was small; tubercle bacilli were seen in groups in small tubercles composed of epithelioid cells and among the peripherally placed nuclei of giant cells. Granules which were not acid-fast were seen within epithelioid cells of many of the tubercles after one week and were regarded as presumptive evidence of destruction of tubercle bacilli. Nevertheless, the occurrence of well preserved and viable organisms long after infection indicates that destruction was not complete. Numerous acid-fast bacilli were seen in epithelioid cells and among the peripherally placed nuclei of giant cells in the lungs of a rat eighteen months after infection

TABLE 7.—*Tubercle Bacilli in One Hundred Oil Immersion Fields of Organs of Rats Infected Intravenously with 1 Mg. of Bovine Tubercle Bacilli*

Rat	Time After Infection	Tubercle Bacilli Counted		
		Lung	Spleen	Liver
43	2 hours	272	19	0
41	2 hours	177	13	0
42	3 days	79	1	1
44	8 days	80	57	47
45	10 days	30	2	24
15	15 days	4	19	14
53*	15 days	10	4	12
M13	19 days	26	12	47
26†	26 days	About 5,000	335	143
M14	29 days	0	6	0
16	31 days	1	14	19
57*	35 days	21	14	6
M10	35 days	0	0	0
M11	42 days	0	0	0
M12	49 days	1	0	0
17	62 days	23	0	0
11*	72 days	48	6	12
8†	73 days	242	0	0
7†	87 days	0	0	0
25*	97 days	..	0	0
23*	147 days	124	12	0
56†	181 days	303	83	13
20†	186 days	About 2,000	0	0
51†	306 days	131	0	8
52†	352 days	About 2,000	..	0
54†	477 days	About 5,000	201	40

* Death was due to the reaction to tuberculin.

† The rat died.

(fig. 2A). Lung tissue from this animal, seeded on Petraghani's medium, yielded abundant growth of tubercle bacilli within one month.

Spleen: Although macroscopic tubercles were never present in the spleen, the organ was enlarged, and microscopic tubercles were present in it in all animals after the first week. Two hours after infection, a few clumps of acid-fast bacilli were found in the terminal arterioles of the pulp, and occasionally acid-fast bacilli were seen in polymorphonuclear or mononuclear cells in the cords of the pulp. From the end of the first week to the end of the fifth week, numerous tubercle bacilli were found in many discrete or rarely confluent groups of epithelioid cells, measuring from 20 to 40 microns across, and situated in the splenic corpuscles and in the pulp cords (fig. 2B). Single necrotic

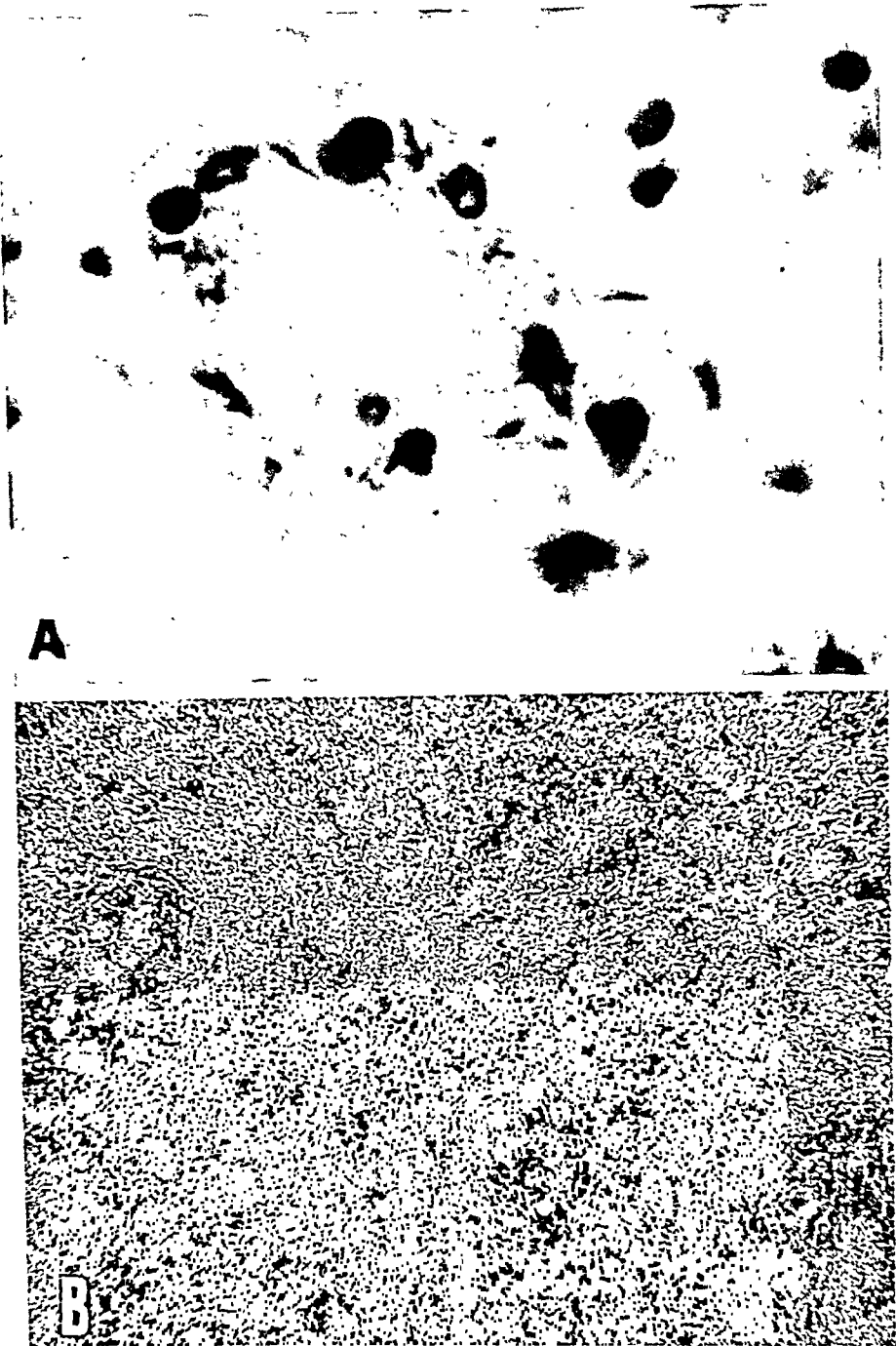


Fig. 2.—*A*, giant cell in the lung of rat 54, which died eighteen months after infection; $\times 1,700$. Numerous tubercle bacilli may be seen among the peripherally placed nuclei. *B*, spleen of rat 44, which was killed eight days after infection; $\times 62$. Numerous small discrete nodules composed of epithelioid cells that contain tubercle bacilli are seen in the red and white pulp. The spleen weighed 2 Gm.

epithelioid cells were seen in many of the tubercles, but caseation was not observed. Many megakaryocytes, as well as numerous mononuclear cells in mitosis, were seen in the splenic pulp. The spleens averaged in weight approximately 2 Gm., the weight of the spleen of a normal rat being approximately 0.5 Gm. From the sixth week to the tenth month, tubercle bacilli were found with difficulty in most of the spleens but were abundant in some. The small collections of epithelioid cells noted in a foregoing statement were slightly reduced in size. Giant cells and fibroblasts were rarely seen. The spleen in this period varied in weight from 0.8 to 2.4 Gm.

From the time of infection up to thirty-five days, tubercle bacilli were demonstrable in all spleens (table 7); after this time in most spleens none was observed. Nevertheless, in animals with exceptionally large numbers of tubercle bacilli in the lungs, the organisms were demonstrable in the spleen. It is noteworthy that yellowish brown lipoid droplets, as well as nonacid-fast granules, occasionally in chains the length of tubercle bacilli, were observed in epithelioid cells of many spleens after the first month.

Liver: Macroscopic tubercles were not found in the liver. Small microscopic collections of epithelioid cells, often containing tubercle bacilli, were seen in the livers of all animals after eight days and were similar to those found in sections of lung and spleen. These simple tubercles occurred within the hepatic lobule and in periportal spaces; their number and size diminished with increasing intervals after infection. After corresponding intervals they were less numerous in the liver than in the lung or spleen. Giant cells were found in the liver of a single animal which was killed two weeks after infection.

Two hours after the injection of 1 mg. of bovine tubercle bacilli, no organisms could be demonstrated in the liver; from three to thirty-five days after infection, they were present in most of the animals. After thirty-five days, they were found only in those rats the lungs of which had shown the micro-organisms in great numbers. Nonacid-fast granules were seen in tubercles of the liver in 2 rats one month and six months after infection.

Kidney: Gross lesions were not found in the kidney. The characteristic tubercles composed of epithelioid cells were seen in the kidneys of 2 rats that were killed ten and thirty-five days after infection. Tubercle bacilli were found in scattered large mononuclear cells in the interstitial tissue of the kidney of a single rat eighteen months after infection.

Tuberculosis in Rats Previously Treated with Heat-Killed Tubercle Bacilli.—Autopsies were made on 13 rats that had been given repeated injections of heat-killed tubercle bacilli and subsequently infected intravenously with 1 mg. of bovine tubercle bacilli. These animals died or were killed from nineteen to one hundred and seventy-two days after

infection. Minute macroscopic tubercles were found in the lungs of all the animals after one month, and conspicuous enlargement of the spleen occurred in every rat. There were histologic tubercles composed of epithelioid cells in the lungs, spleen and liver of every animal. Occasional necrotic epithelioid cells were present in many of the tubercles, but caseation was never observed. Giant cells were seen in the lungs of most rats but were found in the spleens or livers of only a few. The lesions were similar in every respect to those of the tuberculous rats described.

Tubercle bacilli were present in small numbers in the lungs, spleen or liver in 5 immunized and infected rats, and none were found in the organs of 8 animals (table 8). A comparison of tables 7 and 8 shows that fewer tubercle bacilli were demonstrable in the organs of the

TABLE 8.—*Tubercle Bacilli in One Hundred Oil Immersion Fields of Organs of Immunized Rats Infected Intravenously with 1 Mg. of Bovine Tubercle Bacilli*

Rat	Immunization Group	Time After Infection, Days	Tubercle Bacilli Counted		
			Lung	Spleen	Liver
27*	D	19	0	0	0
31*	D	19	0	0	0
M3	B	19	6	14	18
M9	B	29	0	0	0
M8	B	35	0	0	0
M1	B	42	0	0	0
M5	B	49	0	0	0
13†	A	61	53	0	0
2†	A	74	4	19	0
5*	A	97	2	0	0
10*	A	97	0	0	0
14*	A	97	0	0	0
6*	A	172	90	6	3

* This rat died of the reaction to tuberculin.

† This rat died.

immunized than in the organs of the previously normal rats. Organisms were seen in the lungs, spleen or liver in 11 of 15 previously normal rats that were examined between nineteen and one hundred and eighty-one days after infection (table 7). Nonacid-fast granules were present in epithelioid cells in the lungs of all immunized and infected rats and in the spleens or livers of only a few animals.

COMMENT

The experiments reported in the present paper show that in rats in which tuberculosis developed following the intravenous injection of 1 or 10 mg. of virulent bovine tubercle bacilli the skin did not react to tuberculin. Skin reaction to tuberculin also failed to occur after repeated injections of killed tubercle bacilli, even if this treatment was followed by infection with bovine tubercle bacilli. The small nodules that followed injections of heat-killed tubercle bacilli did not increase

in size with repeated injections, as in the rabbit (Opie and Freund ²¹). Tuberculin repeatedly injected into infected rats did not induce cutaneous sensitiveness to tuberculin.

In contrast to the lack of skin reaction to tuberculin, there was systemic reaction in infected rats. Normal rats tolerated 1,000 mg. of tuberculin injected into the peritoneal cavity. Tuberculous rats died after the injection of 20 mg. of tuberculin. It was noteworthy that the site of injection of tuberculin, namely, the skin or the peritoneal cavity, did not seem to influence the systemic reaction. That the rat possesses a high degree of hypersensitiveness is further indicated by the experiments of Freund,²² who found that tuberculous guinea pigs succumb to the intraperitoneal injection of 120 mg. of tuberculin but not to 60 mg. One thousand milligrams of tuberculin injected into 2 rats immunized with heat-killed tubercle bacilli caused general malaise in both. One of these animals died after five days.

Our observations in regard to the systemic effect of tuberculin on tuberculous rats differ from those recorded in the literature. All previous attempts to demonstrate systemic hypersensitiveness to tuberculin in tuberculous rats have failed. M. I. Smith, however, found that tuberculous rats die with tuberculin shock when very large amounts of tuberculoprotein are injected into the blood stream. To explain the difference between the reported observations of others and those described in the present paper, it is noteworthy that the strain of tubercle bacilli used in our experiments is of unusually high virulence (0.00001 mg. injected intravenously kills a rabbit in from three to six months through extensive pulmonary and renal lesions), and the tuberculin employed is of high potency, approximately one and one-half times that of the international standard tuberculin.

The tuberculous rat is highly susceptible to the systemic action of tuberculin although its skin does not react to tuberculin. Cutaneous sensitization in the presence of systemic reactivity does not occur in young tuberculous guinea pigs (Freund; ²² Valtis ²³) and in tuberculous rabbits in the postallergic phase, i. e., when skin reactivity disappears preceding death (Freund, Laidlaw and Mansfield ²⁴). The systemic reaction to tuberculin appears to be specific even though tuberculous tissues are more susceptible to injurious agents than normal tissues (Bordet; ²⁵ Freund ²⁶).

22. Freund, J.: *J. Immunol.* **13**:285, 1927; **17**:465, 1929.

23. Valtis, J.: *Compt. rend. Soc. de biol.* **99**:554, 1928.

24. Freund, J.; Laidlaw, E. H., and Mansfield, J. S.: *J. Exper. Med.* **64**:573, 1936.

25. Bordet, P.: *Compt. rend. Soc. de biol.* **106**:1251, 1931.

26. Freund, J.: *Proc. Soc. Exper. Biol. & Med.* **30**:535, 1933; *J. Exper. Med.* **60**:661 and 669, 1934; *J. Immunol.* **30**:241, 1935.

The only report in the literature on the formation of antibodies against tubercle bacilli in the tuberculous rat is that by Ornstein and Steinbach.⁶ These authors found that complement-fixing antibodies were not produced in rats infected with a human strain of very low virulence (H37). We did not observe formation of antibodies in rats infected with virulent bovine tubercle bacilli. Nevertheless, complement-fixing antibodies were demonstrable in most of the infected rats that received one or more injections of tuberculin. Calmette and his co-workers²⁷ stated long ago that the injection of tuberculin promotes the formation of complement-fixing antibodies in tuberculous human beings.

The formation of antibodies was demonstrable in most of the rats that received repeated injections of heat-killed tubercle bacilli. Antibodies were produced in all of 4 animals that received intraperitoneal in addition to subcutaneous injections of heat-killed tubercle bacilli, and the antibody titer varied from 1 in 40 to 1 in 80.

Our observations show that rats are highly resistant to tuberculosis. For a large dose of highly virulent tubercle bacilli has not been fatal to them although tuberculous lesions, in most instances containing demonstrable tubercle bacilli, have been found in them after death. In these rats tuberculosis has progressed for only a short time and tubercles have not increased in size or number and have failed to caseate. The Royal Commission on Tuberculosis¹³ and Ornstein and Steinbach⁶ stated that tubercle bacilli multiply freely in tissues of the rat without inducing the formation of tubercles. We have found that the tubercle bacilli induce the formation of tubercles and that the micro-organisms are in epithelioid and giant cells. The number of micro-organisms in these cells is moderate in most of the rats, but tubercle bacilli in immense numbers were seen in the lungs of 4 rats that died one, six, twelve and eighteen months after infection. In most instances, though the disease has failed to progress, tubercle bacilli have persisted, and in a few animals they have undergone active multiplication and are found in great numbers within epithelioid and giant cells.

In the so-called rat leprosy, J. Jadassohn²⁸ and Muir²⁹ found enormous numbers of acid-fast bacilli in mononuclear cells. The micro-organisms were so numerous and closely packed that they appeared in

27. Calmette, A.; Massol, L., and Mezie, A.: *Compt. rend. Soc. de biol.* **73**: 122, 1912.

28. Jadassohn, J., in Kolle, W., and Wassermann, A.: *Handbuch der pathogenen Mikroorganismen*, ed. 3, Jena, Gustav Fischer, 1928, vol. 5, p. 1063.

29. Muir, E.: *Leprosy*, in *A System of Bacteriology*, Privy Council, Medical Research Council, London, His Majesty's Stationery Office, 1930, vol. 5, p. 379.

stained sections as red masses filling the cytoplasm. They were also present in giant cells and free in small blood vessels.

It is noteworthy that caseation of tubercles occurs in susceptible animals, such as the guinea pig and the rabbit, that acquire cutaneous sensitization to tuberculin, and is absent in the rat, in which the skin reaction does not occur. Nevertheless, caseation occurs in the tuberculous lesions of the lower monkeys though their skin is said to be refractory to tuberculin.

SUMMARY

Rats infected with highly virulent bovine tubercle bacilli fail to react with allergic inflammation to tuberculin injected into the skin but are killed through tuberculin shock by a small quantity of tuberculin.

Rats given repeated injections of heat-killed tubercle bacilli do not show cutaneous sensitization.

Rats immunized with heat-killed tubercle bacilli and subsequently infected are more susceptible to the systemic action of tuberculin than rats that have not been immunized.

Complement-fixing antibodies are not demonstrable in tuberculous rats, but they appear in infected animals after repeated injections of tuberculin.

Rats that receive several injections of heat-killed tubercle bacilli intracutaneously or subcutaneously show development of antibodies regularly; antibody formation is abundant after intraperitoneal injections.

After intravenous injection of 1 mg. of virulent bovine tubercle bacilli into the rat macroscopic tubercles develop in the lungs, the spleen becomes conspicuously enlarged, and microscopic tubercles are found in the spleen, in the liver and rarely in the kidneys. These lesions after approximately one month do not progress but persist until death. Tubercle bacilli are usually found in moderate numbers in epithelioid and giant cells. In a few instances tubercle bacilli are demonstrable in great numbers in histologic sections and are present within epithelioid and giant cells.

Tuberculosis in rats that have received repeated injections of heat-killed tubercle bacilli is similar to tuberculosis in previously normal animals, but in the former there is greater destruction of tubercle bacilli.

HISTOLOGY OF THE CUTANEOUS REACTION TO BRUCELLA MELITENSIS ANTIGEN

ISADORE GERSH, M.D.
AND
WILLIAM C. BLACK, M.D.
DENVER

In a recent study of the incidence of positive immunologic reactions for undulant fever, agglutination tests were done on 5,000 unselected blood samples, and skin tests were carried out as a routine on 491 of the patients whose bloods were tested.¹

The agglutination and skin tests for undulant fever are extensively used as diagnostic aids. A positive skin reaction is generally believed to indicate bacterial sensitization resulting from past or present infection with *Brucella*.

A review of the literature on undulant fever failed to reveal reports dealing with the histologic picture of the skin reactions in man. For this reason, another series of intradermal tests was carried out, and a specimen of skin for biopsy was removed from each of the persons whose reaction was positive. Tissues were obtained at intervals from forty-eight to one hundred and ninety-two hours after the injection.

PROCEDURE

The antigen used for the intradermal tests was made from a strain of *Brucella melitensis* var. *abortus*, isolated from a patient with acute undulant fever. The strain was grown on a 2 per cent nutrient agar medium for forty-eight hours, suspended in physiologic solution of sodium chloride containing 0.5 per cent phenol, then diluted to a density of 30 (silica standard), which is about one-tenth the density of the usual *brucella* stock vaccine. The antigen was proved to be sterile.

For the test 0.1 cc. of this antigen was injected intradermally into the anterior surface of the forearm, and the reaction was observed forty-eight hours later. A positive reaction at that time showed a tender edematous area at the site of injection, with a central indurated area varying from 1 to 2 cm. in diameter and a peripheral zone of erythema from 2 to 8 cm. in diameter. An elliptic segment of skin measuring approximately 1 by 2 cm. was excised for histologic study from every patient who gave a positive reaction. The skin was removed after 2 per cent procaine solution had been injected into the normal skin around the zone of reaction. The tissue was excised by sharp dissection, and the edges

From the Department of Clinical Pathology and the Department of Pathology, University of Colorado School of Medicine and Hospitals.

1. Gersh, I., and Mugrage, E. R.: *J. Lab. & Clin. Med.* **23**:918, 1938.

of the skin were approximated with dermal suture. Prompt healing by first intention resulted. Cultures of the removed tissue proved sterile.

Specimens of skin were obtained from 12 patients: from 4 at forty-eight hours after injection; from 2 at seventy-two hours, from 2 at ninety-six hours, and from 1 each at one hundred and twenty, one hundred and forty-four, one hundred and



Fig. 1.—*A*, forty-eight hour reaction in the skin, showing infiltration of the derma by lymphocytes and monocytes. *B*, seventy-two hour reaction, showing edema and ulceration of the epidermis with extensive necrosis and leukocytic infiltration of the derma.

sixty-eight and one hundred and ninety-two hours. The specimens were fixed in solution of formaldehyde U. S. P. diluted 1 to 10 and were embedded in paraffin for sectioning. Sections were stained by Gram's method, with hematoxylin and eosin and with pyronin-methyl green.

HISTOLOGIC OBSERVATIONS

The following is a brief description of the changes noted in the histologic sections. The sections are described in groups according to the interval of time at which they were removed.

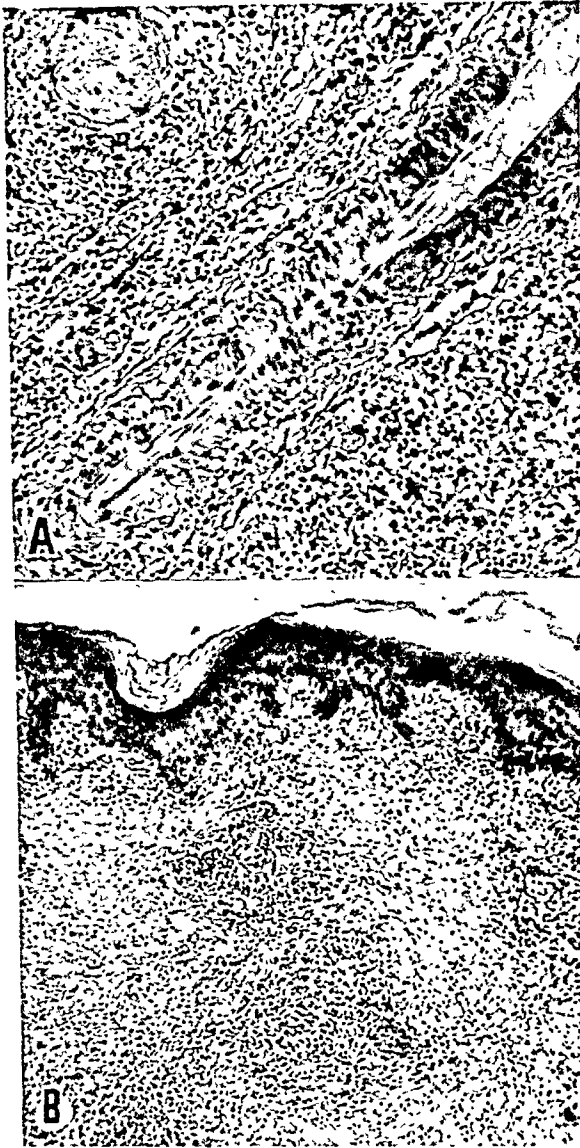


Fig. 2.—*A*, ninety-six hour reaction, showing perivascular infiltration. *B*, one hundred and twenty hour reaction, showing characteristic lymphocyte-monocyte infiltrate in the derma.

In the forty-eight hour specimens the epidermis showed no evident change in 1, from slight to moderate edema in 2 and marked edema with formation of blebs in 1. There was from slight to marked edema of the derma with a cellular infiltrate composed of lymphocytes and mono-

cytes or histiocytes in all 4 (fig. 1 *A*). There was focal necrosis of connective tissue in 2, and in these polymorphonuclear leukocytes were numerous, especially in the areas of necrosis. Gram-negative coccoid bacilli resembling *Br. abortus*, located extracellularly, were present in 1 but were not demonstrable in the other 3. Identification of *Br. abortus* in the remaining sections of the other groups proved to be impossible. In a specimen in which focal necrosis was present several of the larger blood vessels had undergone thrombosis.

One of the seventy-two hour specimens showed the epidermis normal. In the other the epidermis was edematous and showed a central shallow ulcer. In the first specimen there was slight and in the second marked edema of the derma. Both specimens showed dense leukocytic infiltration, which was predominantly lymphoid in the first, while in the second there was a large admixture of polymorphonuclear leukocytes accompanying extensive necrosis of connective tissue (fig. 1 *B*).

One of the ninety-six hour specimens showed normal epidermis except at the site of the needle puncture, where the break in continuity was covered by a thin crust of desiccated fibrin. One disclosed slight edema of the derma; the other, none. Both showed the characteristic lymphocyte-monocyte infiltrate, with polymorphonuclear leukocytes concentrated in areas of necrosis (fig. 2 *A*).

In the specimen obtained at one hundred and twenty hours the epidermis was normal and the derma free from edema. There was an infiltrate of leukocytes, chiefly lymphocytes, but with many monocytes present, some of which were in mitosis. A small zone of necrosis containing polymorphonuclear leukocytes was present (fig. 2 *B*).

In the specimens taken at one hundred and forty-four, one hundred and sixty-eight and one hundred and ninety-two hours edema was slight or absent. The infiltrate was dense and tended to be diffuse. The infiltrating cells were of the same types as in the earlier specimens, but the monocytes were more numerous with occasional nuclei in mitosis. Evidence of phagocytosis of lymphocytes by these monocytes was seen. Large necrotic zones, in which the connective tissue fibers were fragmented, were densely infiltrated by polymorphonuclear leukocytes, and in some instances abscesses had formed. Such zones were bordered by the usual lymphocyte-monocyte infiltrate, with polymorphonuclear leukocytes becoming less numerous toward the periphery.

COMMENT

Certain general features of the reaction in the skin as seen microscopically are common to all the specimens examined. The reaction consists of a cellular infiltrative process, in which there is slight to

moderate edema with collections of cells in the loose areolar connective tissue of the corium around blood vessels, nerves, hair follicles and sweat and sebaceous glands. Where the infiltrate extends into the panniculus adiposus it follows irregularly branching pathways of loose perivascular connective tissue.

The epidermis and the coarse collagenous connective tissue fibers of the derma are not altered in the milder reactions, but in the more severe ones necrosis occurs. Small foci of necrosis are present in the derma as early as forty-eight hours. The epidermis is not the primary site of necrosis but may undergo secondary changes with bleb formation and ulceration.

The tissue reaction following intradermal injection of Br. abortus vaccine may be assumed to vary in intensity with the degree of sensitivity of the tissues and with the amount and concentration of vaccine injected. As the strength of the vaccine used was constant, the difference in severity of the tissue reactions in different persons with the same interval of time is thought to be dependent on the difference in their sensitivity to the brucellas. Apart from the individual difference in sensitivity, the older reaction exhibits more extensive destruction of tissue, with little evidence of resolution, indicating that the reaction is progressive and reaches its height after forty-eight hours or more. In this and in other respects it resembles the reaction to tuberculin as described by Dienes and Mallory.² It differs from the latter reaction, however, in that the necrosis begins in the corium rather than in the epithelium.

The infiltrating cells are predominantly lymphoid, with variable numbers of large mononuclear cells of the histiocyte or monocyte type. Plasma cells are present but not numerous. Giant cell formation is not observed. Hyperemia is not prominent in any of the specimens. In the absence of necrosis polymorphonuclear neutrophilic leukocytes are few and scattered, but where necrosis is present large numbers of these cells occur, collecting in and about the necrotic zone. Eosinophilic leukocytes are not found. The observations of Dienes and Mallory² as regards the tuberculin reaction suggest that the polymorphonuclear reaction is secondary to necrosis of epithelium. The association of these leukocytes with necrosis and their relative scarcity in the tissues showing milder changes due to Br. abortus infection had been noted by Hallman, Sholl and Delez.³

2. Dienes, L., and Mallory, T. B.: *Am. J. Path.* 8:689, 1932.

3. Hallman, E. T.; Sholl, L. B., and Delez, A. L.: *Observations on the Pathology of Bacterium Abortus Infections*, Technical Bulletin no. 93, Michigan State College, Agricultural Experiment Station, 1928.

SUMMARY

The tissue reaction to the intradermal injection of *Br. melitensis* var. abortus antigen in 12 persons giving a positive reaction has been studied histologically. The reaction is inflammatory. When it is mild, it is characterized by infiltration of the derma by lymphocytes and monocytes; when it is severe, it is accompanied by connective tissue necrosis and infiltration by polymorphonuclear leukocytes.

Case Reports

BOTRYOMYCOSIS

Report of Two Cases of Intra-Abdominal Granuloma

PAUL KIMMELSTIEL, M.D., AND PHILIP W. ODEN, M.D., RICHMOND, VA.

We have recently observed 2 cases of granuloma of the abdominal cavity in which botryomycotic granules could be demonstrated.

CASE 1

A colored man aged 33 years was admitted to the hospital division of the Medical College of Virginia three times during 1936, the first time on July 6.

He stated that for three weeks he had had severe cramplike pain just above the umbilicus. The pain radiated to and fro across the abdomen and was worse immediately after eating. Drinking cold liquids caused paroxysms of pain. He noticed a tender mass in the midupper part of the abdomen. The stools became more frequent and were loose and watery. Ten years previously he had received antisyphilitic treatment. He was well developed. There was marked dental caries. The abdomen showed a mass 2 inches (5 cm.) in diameter midway between the xiphoid process and the umbilicus. This disappeared when the abdominal muscles were contracted. It was slightly tender; it was fixed; no sounds were heard over it on auscultation, but it transmitted the aortic pulsations. There was generalized lymphadenopathy.

Roentgen examination revealed a filling defect in the middle portion of the transverse colon, apparently due to an extrinsic mass. A barium sulfate enema showed a sharply defined irregularity of the inferior border of the transverse colon for 2 inches in the midportion.

Urinalysis gave negative results. The red blood cell count was 3,920,000. The hemoglobin content was 67 per cent (Sahli). The white blood cell count was 8,100, with polymorphonuclears 58 per cent and lymphocytes 42 per cent. The Wassermann and Klein reactions of the blood were positive.

Exploratory laparotomy (Dr. H. J. Warthen) in July 1936 revealed an inflammatory mass 5 cm. in diameter arising from the right side of the transverse colon and densely adherent to the round ligament, omentum and jejunum. No obstruction was noted. The stomach and retroperitoneal tissues were free. An appendectomy was done. The abdomen was closed in layers, without drainage. Cultures from the mass showed colon bacilli and *Staphylococcus albus*.

The postoperative course was uneventful. A barium sulfate enema August 3 showed considerable diminution of the obstruction of the transverse colon. The patient was discharged August 5.

He was readmitted August 12 with abdominal pain suggesting a partial intestinal obstruction. He improved under conservative treatment. A barium sulfate enema showed slight irregularity of the transverse colon. He was discharged August 19 in good condition.

From the Department of Pathology, Medical College of Virginia.

He was admitted again August 28, stating that he had noticed a tender swelling in the lower part of his incision for twenty-four hours. There was a rather firm nonfluctuant tender mass in the lower portion of the old incision, to the right and just above the umbilicus. Two days later an abscess in the abdominal wall was emptied, 2 ounces (59 cc.) of pus being obtained. A culture revealed *Staphylococcus*.

An acute mechanical intestinal obstruction developed. September 18, a laparotomy (Dr. H. J. Warthen) showed the transverse colon to be bound into a dense inflammatory mass with three loops of small intestine. There was marked constriction of these loops. The mass was excised with V-shaped areas of the small intestine and colon. A colostomy through a McBurney incision on the right side was done. The wound was closed in layers, with drainage. A fish bone 1 inch (2.5 cm.) long was found in the midst of the mass.

Massive atelectasis of the right lung developed. A bronchoscopic aspiration was done September 22 for the atelectasis, with little improvement. The temperature curve was of a septic type. The patient received two transfusions and supportive treatment. The wound became grossly infected. The condition became progressively worse, and the patient died October 12. Permission for a post-mortem examination was refused.

The mass removed at the last operation, measuring 7 by 3.5 by 2.5 cm., was attached to a piece of large intestine. It was firm in consistency, and its cut surface was light gray and homogeneous. The center of the mass contained a few well defined soft areas, which were rather dark red with ochre yellow centers (fig. A, left).

Histologically, the mass consisted of a dense acellular connective tissue with scanty vessels and in several places hyaline degeneration. A few scattered areas of perivascular round cell infiltration were noted. Within the areas there was a rather cellular granulation tissue composed of fibroblasts and numerous large mononuclear foam cells, numerous foreign body giant cells and an irregular infiltrate of lymphocytes and plasma cells. Russell's bodies were frequently encountered. Polymorphonuclear cells, however, were almost absent. The areas were rather vascular. Although there was no evidence of recent hemorrhage, the presence of numerous iron-pigmented macrophages indicated old hemorrhage. The tissue contained several small, well circumscribed abscesses with complete liquefaction of tissue and dense infiltration with polymorphonuclear cells. In the centers of some of these abscesses, granules were found (fig. B). Under low power magnification they resembled the "sulfur granules" in actinomycosis. With the hematoxylin and eosin stain they were rather dark blue and irregularly finely granular, within dense dark bluish borders. These granules were surrounded by a narrow pinkish-stained hazy zone, which in some places was more condensed and formed clublike excrescences. A doubly refractive membrane was absent. The Gram stain showed that the granules contained numerous gram-positive cocci, which were concentrated at the borders, although they were not found within the clublike excrescences themselves. The cocci were mostly arranged in rather long chains resembling streptococci. In some places they were clumped together. No cocci were demonstrable outside the granules. The matrix of the granules did not take the Gram or a fibrin stain. The centers of most of these granules stained reddish with the Kernechtrot¹ counterstain.

1. The formula of the Kernechtrot counterstain is as follows: One-tenth gram of Kernechtrot (obtainable from Pfaltz and Bauer, New York) is dissolved in 100 cc. of 5 per cent solution of aluminum sulfate by boiling. The sections are stained for from two to five minutes and washed in water without further differentiation.

CASE 2

A white woman 51 years of age was admitted to the hospital division of the Medical College of Virginia, to the surgical service, Oct. 19, 1936, because of a mass in the right lower quadrant of the abdomen, which had been present for five or six months. At times this mass caused considerable pain. The woman had complained of chronic constipation over a period of years. She had no diarrhea, no blood in the stools and no digestive disturbances. She was totally deaf. Her physical condition was good except for a firm rounded mass in the right lower quadrant of the abdomen, apparently fixed to the inner surface of Poupart's ligament. It was approximately 3 cm. in diameter. Roentgenograms of the pelvis showed nothing of importance except slight hypertrophic arthritis of both hip joints.

Urinalysis gave negative results. The red blood cell count was 3,900,000. The hemoglobin content was 78 per cent (Sahli). The differential count showed 72 per cent polymorphonuclear leukocytes and 28 per cent lymphocytes. The Klein and Wassermann reactions of the blood were positive.

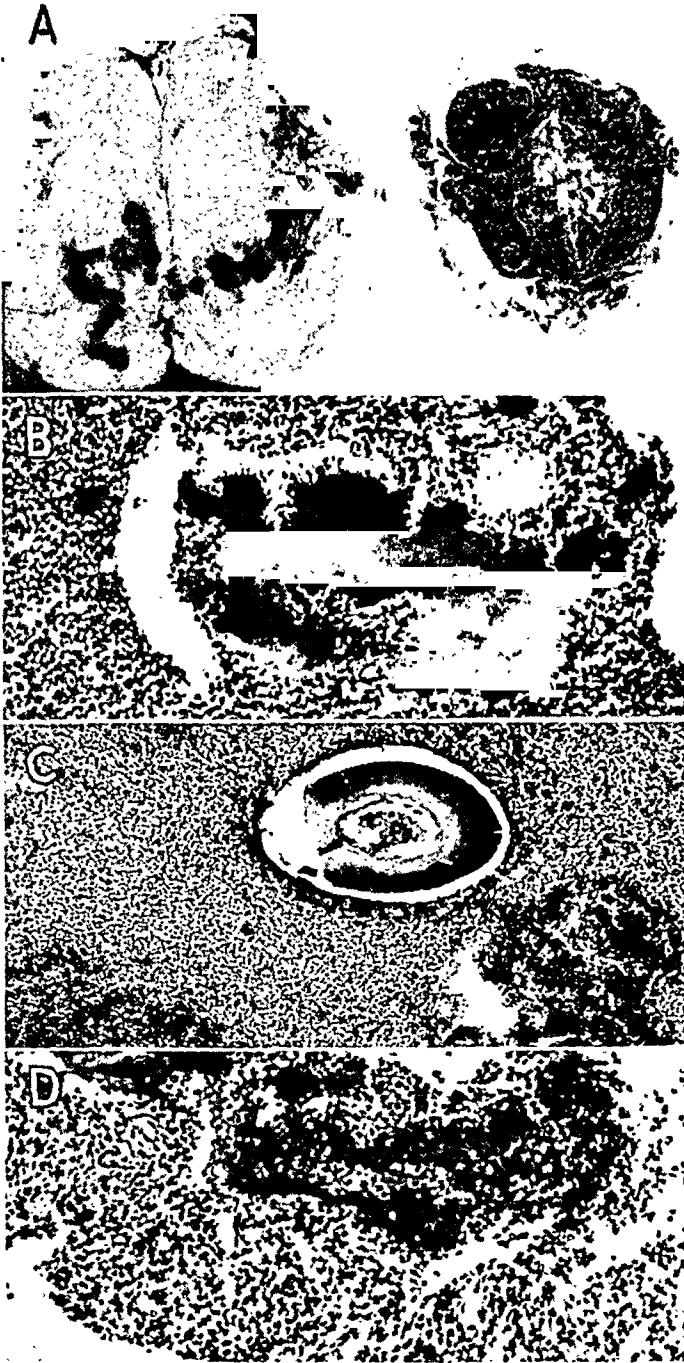
At operation a low incision of the right rectus muscle was made, and the omentum was found adherent to the parietal peritoneum. A mass was present involving the peritoneum and the internal and external fascial sheaths of the rectus muscles. The entire mass was excised within a block of the abdominal wall. A fish bone was found in the mass. The abdomen was closed in layers, without drainage. The patient made an uneventful recovery and was discharged completely healed on November 4.

The gross specimen measured 5.5 by 2.5 by 2 cm. On the cut surface it was homogeneously gray except for a few small ill defined reddish-colored areas (fig. A, right). The histologic appearance was that of a dense fibrous tissue with an irregular infiltrate of small round cells. In the majority of blocks the tissue was acellular. In some of them, however, were areas of a cellular granulation tissue. Foreign body giant cells were scattered throughout the entire mass. In one of the areas a fragment of a fish bone was found, which was impregnated with iron salts (positive Perl reaction). There were no polymorphonuclear cells around the fish bone (fig. C). In its vicinity were several small foci in which there was a peculiar degeneration with the nuclei of the fibrous connective tissue densely crowded together, greatly swollen and vacuolated, so that they presented a honeycomb appearance. The paraplasmic substance apparently had undergone liquefactive necrosis, and the entire focus under low power magnification was irregularly dark bluish. There was no polymorphonuclear cell reaction within or in the vicinity of such foci.

In two places, however, the bluish-stained mass of necrotic tissue was condensed and formed clearly delineated granule-like bodies stained dark blue with hematoxylin and eosin and separated from the surrounding tissue by a rather narrow zone of polymorphonuclear cells (fig. D). These granules were not as sharply demarcated as those in case 1; their borders were much more heavily stained than their centers. No clublike excrescences and no membrane could be demonstrated. The Gram stain revealed that the entire mass was free from cocci except the areas of necrobiosis described. Staphylococci were scattered within these foci. The granules themselves, which were surrounded by polymorphonuclear cells, revealed a few staphylococci peripherally.

PATHOGENESIS OF BOTRYOMYCOSIS

This condition, although frequently encountered in the practice of veterinary medicine, as castration fungus in horses, is rarely mentioned as having been observed in man. Six cases of the disease in man seem



A, gross appearance of the two inflammatory tumors (right, case 1; left, case 2). *B*, granule within an area of suppuration in case 1; hematoxylin and eosin stain. Delicate raylike excrescences can be recognized at the border. *C*, fish bone in case 2. The dark stained area is composed of densely crowded swollen nuclei without polymorphonuclear cell infiltration. *D*, condensing mass of debris within an area of suppuration—beginning formation of a granule. Bacteria are found only within this area (case 2).

to be known. Reference to 5 of them, including their own, is found in the paper of Berger and his co-workers.^{1a} The sixth case was reported by Plaut.²

Botryomycosis is a chronic inflammatory lesion in which peculiar granules are found, the nature of which was first determined by Magrou.³ Under low power magnification these bodies resemble the sulfur granules in actinomycosis because of their clublike excrescences. They are situated in small foci of suppuration. The granule itself consists of an unidentified matrix, probably a bacterial and tissue débris, in which bacteria can be demonstrated. The latter are usually crowded in the periphery, and the granule in its characteristic form is surrounded by a doubly refractive membrane. Magrou showed that the lesion is caused by staphylococci.

In all cases except Berger's it has been reported that foreign bodies participated in the development of botryomycosis. In the castration fungus of horses, hairs are found. Bony sequestrums and fish bones are present in the lesions in man. It is, however, still in question whether and how much the foreign body itself contributes to the specific character of the condition. Magrou stated that the lesion can be reproduced in animals in the absence of foreign bodies by inoculating staphylococci, provided the quantity is small enough not to give rise to suppuration and large enough to initiate a slow development of granulation tissue. To our knowledge, his experiments have not yet been reproduced. The fact, however, can hardly be neglected that in 7 (including both our cases) of 8 instances in men foreign bodies were present.

It is furthermore questionable whether in cases of spontaneous botryomycosis in man the limited number of bacteria should be regarded as the main factor in the development of the lesion. Their invasion and multiplication depend largely on the tissue reaction, which therefore must be taken into consideration. Berger and his associates discussed the possibility that it is the type of tissue which inclines to such a reaction. This, however, appears to be unlikely since botryomycosis is found in the scrotal tissue of horses and in the peritoneal, bony and genital tissues of human beings. These authors suggest further that colon bacilli, which in their cases were found together with staphylococci, may have secreted antiphagocytotic substances thus controlling tissue necrosis and reaction. This explanation, however, does not apply to instances in which staphylococci only are found.

The type of bacteria or their virulence does not seem to be important. Magrou was able to obtain identical results in his experiments with ordinary staphylococci as well as with those isolated from spontaneous botryomycosis. Colon bacilli were found to accompany the staphylococci in the case observed by Berger and in our case 1.

ANALYSIS OF OUR CASES

In our first case the bacteria were arranged in scanty, scattered granules which more or less had the characteristics of what is called

1a. Berger, L.; Vallee, A., and Vezina, C.: Arch. Path. **21**:273, 1936.

2. Plaut, A.: Arch. Path. **23**:602, 1937.

3. Magrou, J. E.: Les grains botryomycotiques: Leur signification en pathologie et en biologie générales, Thesis, no. 267, Paris, Laval, 1914.

botryomycosis. It is questionable, however, whether the second case can be identified as one of botryomycosis. In fact, our histologic observations are not compatible with the present concept of this condition. The granules were not surrounded by a cuticula nor were they garnished with clubs. They did, however, bear a great resemblance to those of botryomycosis in that they consisted of a rather homogeneous mass of *débris* which contained cocci. The borders were deeply stained with hematoxylin and sharply delineated, and the granules were embedded in discrete foci of suppuration. The identity of both cases, furthermore, is based on the fact that the lesions were similar in their clinical development and were grossly alike, both containing a fish bone. In both cases, staphylococci were found in discrete areas of *débris*.

We assume that in our second case an early stage of the formation of granules was demonstrated. The first change we observed was that of degeneration of rather dense connective tissue in discrete areas. This degeneration was probably due to action of bacteria, which were found exclusively within such foci. The intercellular material appeared to liquefy, and the nuclei of the fibroblasts underwent marked vacuolar disintegration and swelling. They crowded together, and the chromatin material diffused through the nuclear membrane, which finally disintegrated. The irregularly bluish-stained focus was at first ill defined but with increasing condensation became more sharply bordered. The bacteria remained alive within this shrinking ball of *débris*, and there was remarkably little cellular reaction until the granule was fairly well formed. It was only at this period of development that a polymorphonuclear cell exudate commenced to surround the finally completely detached granule, which then appeared to float in pus.

COMMENT

Although the sequence of events can be demonstrated well in one of our cases, there was nothing that explained clearly why the multiplication of bacteria in botryomycosis is limited to growth within granules. Several factors may be considered: In the first place, the granule may be surrounded by a cuticula. Masson⁴ explained the process in the presence of bone spicules as follows: The staphylococci multiply in the shelter of haversian channels. As soon as the bony substance is destroyed and the clusters of bacteria come in contact with the surrounding tissue, a membrane precipitates.

In the absence of bone protection, however, a different mechanism has to be assumed. Berger and his co-workers mentioned the possibility that the cuticula may result from coagulation necrosis of connective tissue fibers and that staphylococci multiply within its shelter, thickening the membrane by internal apposition of protein derived from dead cocci. Since the membrane itself is described as hard and breakable under the knife, it is unlikely that further enlargement of granules could occur. Thus, the growth of granules is terminated by the precipitation of such a membrane. In the early phases of development, however, the membrane must be of a more plastic nature, and Berger and his associates described it as fibrin-like, although it does not show specific staining properties. They interpreted such membranes as "probably beginning

4. Masson, P.: *Lyon chir.* 15:230, 1918.

shells." It therefore appears to us that the characteristic doubly refractive hard membrane rather represents a terminal phase in the development of the granules and that little is known about the time of its appearance or of its nature in the early stages.

Not all such granules, however, are surrounded by a distinctly visible doubly refractive membrane. Plaut stated that in his case the periphery of each granule was formed by a homogeneous mass obviously consisting of coalescing dead cocci. The observations in our first case are identical with his, but the granules in our second case did not reveal a membrane; the growth of bacteria had been limited to discrete areas without the formation of a mechanical visible barrier.

An adequate explanation of this phenomenon cannot be given yet. It does not appear to be determined by the type of bacteria only, since staphylococci and staphylococci mixed with colon bacilli have been found, nor is the bacterial growth limited by the precipitation of a membrane, since the latter may be absent. We are of the opinion that more emphasis should be laid on the tissue reaction itself, since in the majority of cases foreign bodies have played a role. This suggests that tissue thus irritated differs in its defense mechanism from virginal tissue.

Finally, it should be mentioned that the clublike excrescences may be absent as in the spontaneous lesions of horses. They may vary in number, some of the granules being bare, others provided with clubs. Hence we conclude that both the doubly refractive membrane and rays or club formations are of an accessory nature.

SUMMARY

Two cases of intra-abdominal granuloma are presented, in both of which the granuloma contained botryomycotic granules. The early phases of the development of the granules are described. It is emphasized that foreign bodies play an important rôle in the pathogenesis of this lesion.

MEDIAL DEGENERATION IN A NONRUPTURED AORTA APPEARING SYPHILITIC MACROSCOPICALLY

ANTONIO ROTTINO, M.D., NEW YORK

Medial degeneration has to date been described largely in cases of spontaneous rupture of the aorta. This paper records an instance of severe medial degeneration in which the aorta dilated but did not rupture. It will serve, further, to emphasize that in some instances gross changes may occur which to the naked eye appear like those of syphilis. This real pitfall was alluded to by Erdheim,^{1a} who stated that for some time in demonstrating the lesions of syphilis he had used material which to his amazement the microscope later showed to be nonsyphilitic. This occasioned two reports in which he described a new disease, "medio-necrosis idiopathica cystica."^{1b}

REPORT OF A CASE

A 70 year old woman entered St. Vincent's Hospital, in the medical service of Dr. Thomas A. Martin, Sept. 19, 1936, with congestive heart failure. Her symptoms appeared six months before and increased in severity until she was edematous, dyspneic, and orthopneic at rest. After two months' rest in bed and treatment with digitalis she recovered sufficiently to get up. In three weeks, however, she was again seized with congestive heart failure and therefore entered the hospital.

She was dyspneic and orthopneic, with cyanosis of the mucous membranes and pronounced edema of the lower extremities. The pupils were equal and active. The veins of the neck were dilated. Moist rales were heard at the bases of the lungs. Percussion showed the heart to be enlarged. The sounds were poor; no murmurs were heard. The rhythm was irregular and the rate rapid, 158 beats per minute. The blood pressure was 134 systolic and 78 diastolic. The Kahn test of the blood was negative. The blood sugar was 111 mg. per hundred cubic centimeters. The urine contained albumin (1 plus). An electrocardiogram revealed auricular flutter with varying degrees of block and occasional premature ventricular contractions.

After five days of increasing failure of the heart, the patient died.

Necropsy.—The examination was made twenty-four hours post mortem.

The heart lay free in the pericardial cavity, was generally enlarged and weighed 415 Gm. All the chambers were moderately dilated, while the ventricles were in addition hypertrophied. No unusual valvular changes were noted. Beneath the aortic valve several endocardial pockets were found on the interventricular septum despite normal cusps and commissures. The coronary ostia were wide and fully patent, while the arteries leading from them were thick, tortuous and calcified but unobstructed.

From St. Vincent's Hospital.

1. Erdheim, J.: (a) Virchows Arch. f. path. Anat. **273**:454, 1929; (b) **276**: 187, 1930.

The ascending aorta was transformed into a diffusely dilated, nonelastic, thin-walled sac (12.5 cm. in circumference) which ended abruptly in a moderately dilated arch. The descending aorta was likewise moderately dilated. The intima lining the aneurysm was thick, gray and wrinkled, containing numerous pearly plaques, giving it the appearance of tree bark so often seen in syphilis. The usual yellow of the underlying media was either entirely absent or obscured by the opaqueness of the thickened intima. Beyond the aneurysm the intima for the most part was thin and transparent, so that the underlying, grossly intact yellow media was easily observed. Atheromatous plaques were scattered about the arch as well as around the mouths of the large branches arising from the abdominal aorta. Some were calcified and others ulcerated. The lower portion of the thoracic aorta and the abdominal aorta were entirely smooth.

Other findings were as follows: There was a moderate amount of edema of the legs, with 200 cc. of fluid in the abdominal cavity and 1,000 cc. in the pleural sac, the latter causing some compression atelectasis of the lungs. Two small infarcts were observed in the lower lobe of the right lung. The liver, though not enlarged, presented the nutmeg markings characteristic of chronic passive congestion. The ovaries were sclerotic and the uterus atrophic. The kidneys together weighed 315 Gm. Their surfaces were finely granular. In one kidney two small infarcts were found.

Anatomic Diagnosis.—The diagnosis was: aneurysm of the ascending aorta, probably syphilitic (this was the diagnosis made at the autopsy table; later, when the slides were seen, the diagnosis was changed to medionecrosis idiopathica); calcification and sclerosis of the coronary arteries; bilateral pleural effusion; infarcts of the lower lobe of the right lung; compression atelectasis of the lower lobes of both lungs; chronic passive congestion of the liver and spleen; arteriolonephrosclerosis; infarct of the kidney, and senile atrophy of the uterus and ovaries.

*Microscopic Observations on Aorta.*²—In general, the intima appeared as a wavy, variably thickened layer, here thin, there thick. The thickened portions were in the main composed of dense hyalinized fibrous tissue, in which were intermingled varying amounts of fine elastic fibrils and collagen. In some areas large quantities of calcium were deposited, while here and there were scattered a few muscle cells.

In addition to exhibiting diffuse thickening, the intima contained raised plaques. Most of them consisted of dense fibrous tissue overlying lipoid cavities. Others were entirely fibrous, calcified and even ossified. Beyond the aneurysm the intimal changes were not pronounced.

The continuity of the internal elastic lamina in the ascending aorta was frequently interrupted. In some regions it stood out thick and deeply stained, while in other places it grew pale and disappeared. In some areas where it was absent it became difficult to decide where the thickened intima ended and the media began.

2. The entire length of the ascending aorta was sectioned into serial blocks. The sections extended from just below the aortic valve ring to the beginning of the arch. In addition, shorter transverse blocks were cut from the arch, thoracic aorta and abdominal aorta. They were fixed in solution of formaldehyde U. S. P. and embedded in paraffin, and sections were stained with hematoxylin and eosin, Weigert's stain for elastic tissue, Van Gieson's stain for connective tissue, Mallory's phosphotungstic acid-hematoxylin stain and the Foot and Foot stain for reticulum (Am. J. Path. 8:245, 1932).

The media presented the principal changes, for it had become markedly altered. Histologically, the lesions varied considerably. They were most conspicuous toward the center of the aneurysm, where the media was distinctly thinner than normal, being sandwiched in between the thickened intima and adventitia. The

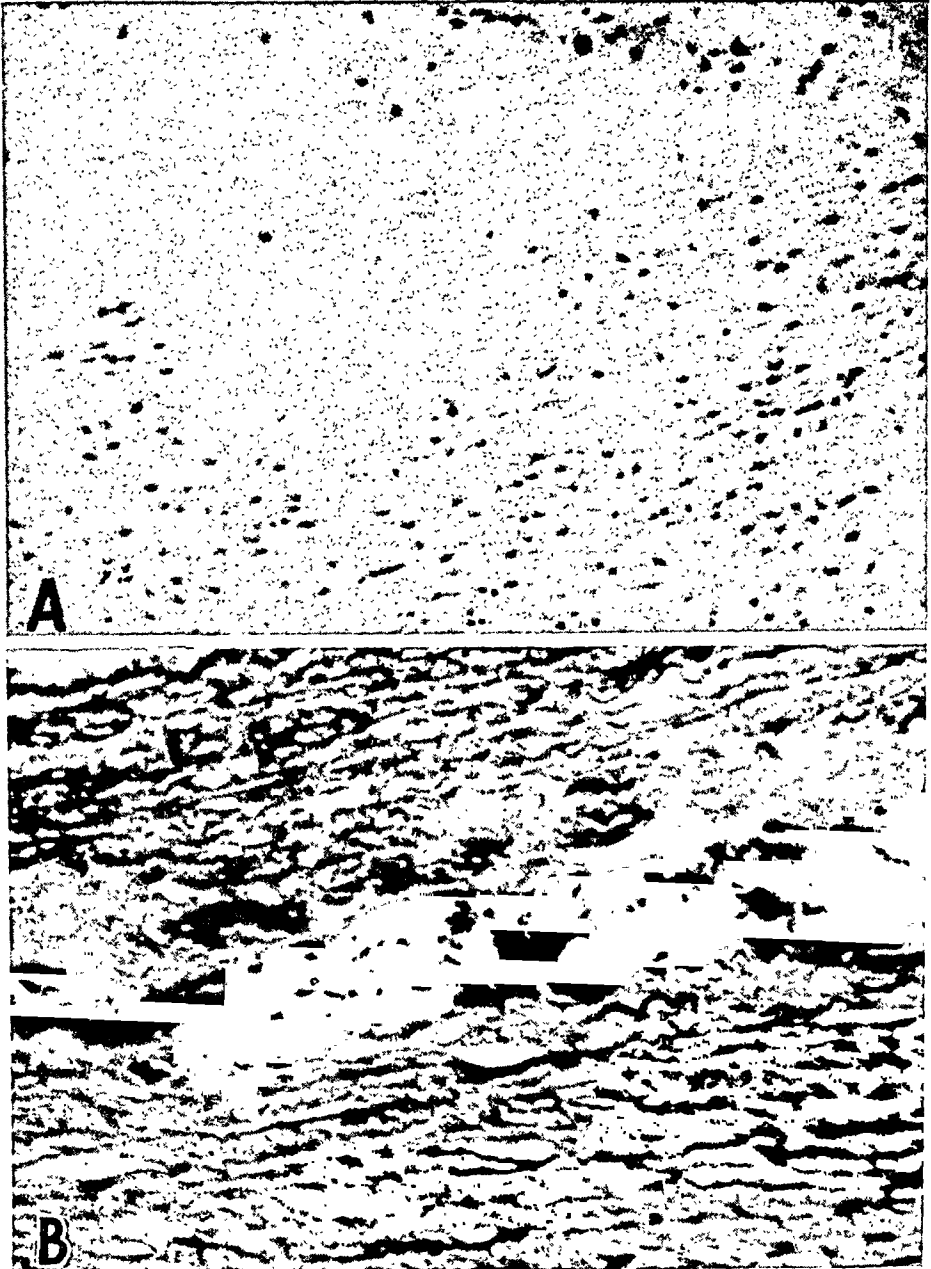


Fig. 1.—*A*, low power magnification of an area of medionecrosis (Gsell³); hematoxylin and eosin. Note focal loss of muscle cells. A few pyknotic nuclei remain. The surrounding muscle cells appear normal. *B*, low power magnification of an area of medionecrosis; Weigert's stain for elastic tissue. Though muscle cells are gone, the elastic elements remain, densely stained, thick, compressed, obliterating the interlamellar spaces.

changes were less pronounced at the aortic root and arch, which corresponded to the periphery of the aneurysm. They were absent in the descending portion of the aorta.

One type of lesion was particularly conspicuous (fig. 1 *A*), as many as twenty-seven lesions of this type being counted over a strip 7 cm. long. They were



Fig. 2.—*A*, low power magnification of irregular areas devoid of elastic laminae; Weigert's stain for elastic tissue. At the margin the elastic laminae end abruptly. *B*, low power magnification of the contents of the defects illustrated in *A*; hematoxylin and eosin. Numerous muscle cells are seen, compactly and irregularly arranged. Sometimes elastic and collagen fibrils are also present.

distributed from one end of the aneurysm to the other, in one direction, and about the entire circumference, in the other. The portion of the media involved varied. Most affected was the midportion, next the outer third and least affected was the inner third. The lesions varied in size, some occupying a small portion

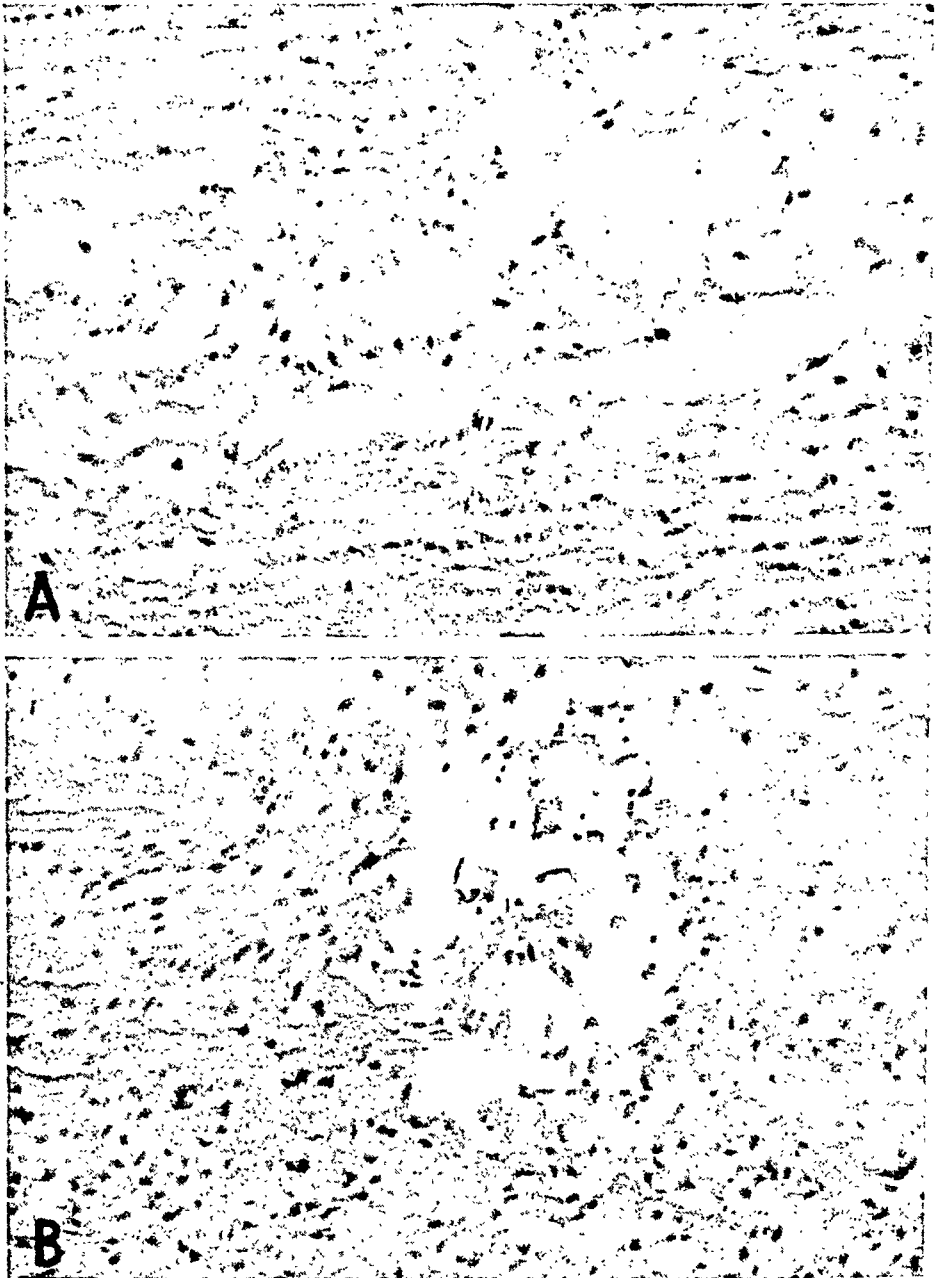


Fig. 3.—*A*, low power magnification of a medial defect from which the elastic tissue is gone but which contains a few muscle cells widely dispersed by mucoid material; hematoxylin and eosin. *B*, low power magnification of a mucoid cyst containing muscle cells; hematoxylin and eosin.

of a low power field, while others measured 3 mm. in length. They involved as much as one-third to one-half the thickness of the media. With the hematoxylin and eosin stain they stood out as deeply eosinophilic areas, devoid of almost all

muscle cells. In a few of these areas nuclei were still present, either pale and swollen or small and pyknotic. With the elastic tissue stain (fig. 1 *B*) the elastic lamellae were seen deeply stained, thick, straight and crowded together so that the interlamellar spaces were much narrowed and the collagen compressed, as a result of which the latter frequently stood out plainly when stained with Van Gieson's stain for connective tissue or Mallory's phosphotungstic acid-hematoxylin stain. The reticulum which normally hugs the lamellae persisted. In an occasional lesion the elastic laminae were fragmented and pale staining.

A second type of lesion observed (fig. 2 *A*) was just as conspicuous, with approximately the same distribution. It was seen to best advantage in sections treated with Weigert's stain for elastic tissue, in which it appeared as irregular gaps at the margins of which the elastic laminae ended abruptly. In sections stained with hematoxylin and eosin (fig. 2 *B*) it was recognized by the large number of muscle cells present, arranged in planes different from that of the normal cells in adjacent areas; i. e., their long axes pointed obliquely or perpendicularly to the plane of the normal cells. In addition, there were occasional areas in which scant amounts of collagen and elastic tissue fibrils intertwined about these cells. In some lesions (fig. 3 *A*) the cells were less numerous and less compactly arranged, being separated by varying amounts of mucoid material. In one field the amount of the latter was considerable, forming a cystlike space in which were suspended remnants of old media (fig. 3 *B*).

The adventitia in general was thick and composed of dense hyalinized connective tissue. The blood vessels were not increased in number and for the most part were small and unchanged. Only a few exhibited mild intimal thickening. Scattered throughout the adventitia were collections of lymphocytes. In one area only were any present in considerable numbers. They were chiefly located in the outermost portion of the adventitia, bearing no relationship to the vasa vasorum.

COMMENT

The pertinent finding in this case is the occurrence in a 76 year old woman of a marked aneurysmal dilatation of the ascending aorta, with wrinkling and puckering of the intimal surface identical with that seen in syphilis. Histologic study showed, however, that the lesion was instead one described by Gsell,³ Erdheim¹ and others in cases of spontaneous rupture of the aorta.

The lesions as seen in the case reported here were focal and characterized by absence of one or more of the normal components of the media. In one lesion there was loss of muscle; in a second the muscle cells were unusually plentiful but the elastic lamellae had disintegrated. Then there were other areas in which abnormal amounts of mucoid material separated the medial elements. Inflammatory reaction and fibrous tissue repair were characteristically absent.

The pathogenesis of the disease is imperfectly understood. It is to Gsell that one owes some of the knowledge of this subject. He felt that the initial lesion was focal necrosis of muscle cells, subsequent to which the remaining components in the same area, without aid of cellular reaction, disintegrated. Following this the defect was repaired by a tissue composed of scant, loose collagenous fibers and a few elastic fibrils. A blood vessel might or might not grow into the area.

3. Gsell, O.: *Virchows Arch. f. path. Anat.* **270**:1, 1928.

Thus the process appeared to consist of three steps—first, necrosis; second, humoral dissolution, and last, repair by an imperfect type of scar. Erdheim amplified the theory by describing more completely the picture in the second stage—the stage of dissolution. He felt that he could demonstrate as a result of the latter process spaces or cysts filled by fluid which stained metachromatically with thionine and cresyl echt violet. To the stage of healing he further added an alternative to Gsell's "imperfect scar," namely, regeneration of muscle. In his opinion, the defects left in the wake of dissolved necrotic areas might become filled again by a regeneration of all the original components, particularly muscle, with this difference, that the normal medial pattern was not reproduced. Instead, the muscle cells were laid down in various positions, with the elastic tissue forming a loose web about them. Finally, he felt that in addition to primary necrosis of muscle the media could undergo destruction by increasing accumulations of mucoid between the lamellae. With confluency of these small collections of mucoid and concomitant disappearance of muscle and thinning of the elastica, mucoid cysts might form.

Much that was described by these two men was seen in the case reported here. Areas of loss of muscle cells were numerous. Unlike the observations in Gsell's cases, however, the anuclear necrotic cytoplasmic remnants of muscle cells were not evident. Also present were areas of the type interpreted by Erdheim as examples of regenerated media. Scar tissue repair as described by Gsell was absent.

As stated medionecrosis has been described chiefly in cases of spontaneous rupture of the aorta. Reference to its presence in intact vessels are few. Weise⁴ studied 120 aortas obtained at necropsies and found medionecrosis in the sense of Gsell in 9 of them. In each the lesion was microscopic and did not lead to macroscopic alteration of the wall. Cellina⁵ selected 10 aortas with a minimal amount of atherosclerosis from persons over 72. In 9 he found focal loss of muscle cells but of a type which he felt was different from that described by Gsell. To the lesion which he observed he appended the name "medionecrosis disseminata"; to Gsell's, "medionecrosis idiopathica." Moritz⁶ alluded to the presence of macroscopic mucoid cysts in several intact aortas.

Another unusual observation in this case was that of intimal change. In reporting aortas which ruptured spontaneously and exhibited medionecrosis, all authors have made it a special point to emphasize the absence of intimal reaction. This lack may have been one of the factors responsible for the ruptures in those aortas and the presence of this reaction in the instance which I have described may explain the failure of the aorta to rupture. Adventitial thickening beneath areas of medionecrosis was described by Gsell. In the case now reported it was likewise present to a marked degree over the entire extent of the aneurysm.

4. Weise, W.: Beitr. z. path. Anat. u. z. allg. Path. **93**:238, 1934.

5. Cellina, M.: Virchows Arch. f. path. Anat. **280**:65, 1931.

6. Moritz, A. R.: Am. J. Path. **8**:717, 1932.

The causes of the disease are entirely unknown. What theories have been advanced are discussed by Shennan.⁷

SUMMARY

A case of aneurysm of the aorta macroscopically mistaken for syphilis but microscopically analogous to the disease described as medionecrosis is presented. The aneurysm occurred in a 76 year old woman, who died in congestive heart failure.

The case is unique since in other recorded instances of advanced medionecrosis the aorta ruptured spontaneously whereas in this case it remained intact.

7. Shennan, T.: *Dissecting Aneurysm*, Medical Research Council, Special Report Series, no. 193, London, His Majesty's Stationery Office, 1934.

AN UNUSUAL FREE BODY IN THE TUNICA VAGINALIS TESTIS

NORMAN ROSENBERG, M.D., NEW YORK

There has been no recent comprehensive review of the literature on the subject of the free body of the tunica vaginalis testis. Schmidt¹ in his paper on the broader subject of free bodies in serous cavities covered some of the older papers on the subject, and Ritter² gave a fairly extensive review of the theories that have been advanced as to the origin of these bodies. The opportunity of describing a specimen of a free body unusual to this serous cavity has therefore also afforded me the privilege of briefly summarizing the knowledge in the field.

REPORT OF A CASE

A 55 year old white man had on the right side a reducible inguinal hernia of fifteen years' duration. Two days before admission he noticed that the mass could no longer be reduced and experienced pain on pressure over this area. The pain was not relieved by local cold applications or rest in bed.

There was a fluctuant swelling extending from the right anterior superior spine into the scrotum and filling the entire inguinal canal. In the canal was a hard mass, about 2.5 cm. in diameter, and a similar mass could be palpated in the scrotum. Because of the accumulation of fluid in the sac, a definite impulse on coughing could not be felt. The hydrocele of the tunica, extending along the inguinal canal, could not be compressed into the abdomen, suggesting that the internal ring was closed. The preoperative diagnosis was irreducible congenital hernia, undescended testicle and hydrocele of the tunica vaginalis.

At operation a sac 18 cm. long was found containing two segments of omentum, which had undergone torsion. One was deep purplish; the other was hyperemic. The testicle was found at the external ring. The fluid content of the hydrocele was clear and of an amber color. Lying free in the scrotal sac was a regularly oval smooth hard mass.

The postoperative course was uneventful, and the patient left the hospital thirteen days after the operation.

Gross Appearance of Specimen.—The specimen consisted of a hernial sac with a rather small and soft testicle and epididymis, together with a small portion of spermatic cord. There also were some small pieces of omentum showing partial fibrosis. The free body was like hard rubber in consistency, ovoid, measuring 2.8 by 2.6 by 2.2 cm., and of a pale yellowish green, partly light red color. It was hard to cut. In its center a flat ovoid smooth-walled cavity, 1 by 0.5 by 0.5 cm., was situated. This was filled with a light ochre-colored homogeneous and rather soft mass.

From the Department of Pathology, Beth Israel Hospital.

1. Schmidt, G. B.: München. med. Wchnschr. 80:410, 1933.

2. Ritter, L.: Deutsche Ztschr. f. Chir. 182:308, 1923.

Microscopic Appearance.—The free body consisted of a densely packed, almost homogeneous-appearing substance, which at the cut edges separated into parallel layers averaging 4 microns in thickness. The many lancet-shaped clefts which were seen in this substance were obviously artefacts. This material did not take any nuclear stain; it became pale red with eosin and held the fibrin stain rather tenaciously.

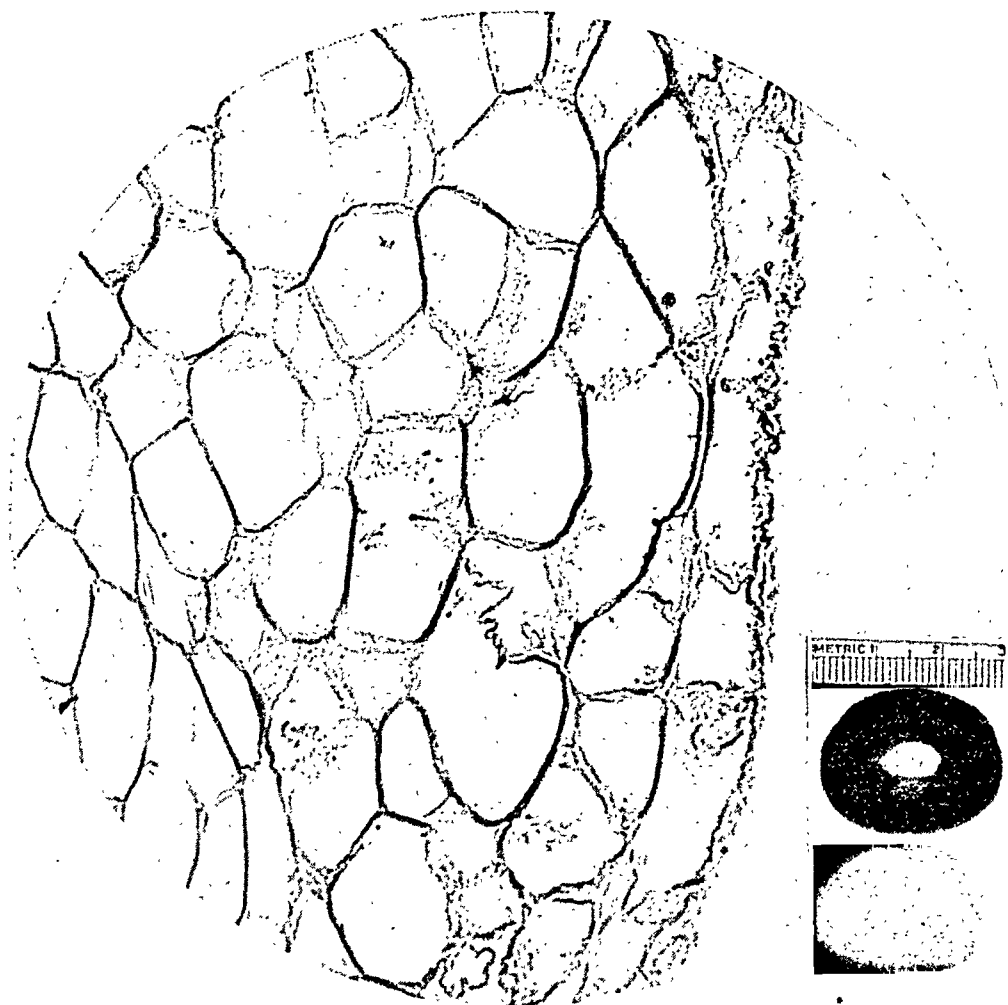


Fig. 1.—The inset shows the free body halved lengthwise. Note the central cavity. The concentric structure is not recognizable. The rest of the figure shows fat tissue from the center of the free body. The structure in general is well preserved.

The brownish soft material from the center was fat tissue, the network of which was well preserved. Occasionally nuclei could be detected, and some erythrocytes were seen in the capillary spaces. The testicle gave the characteristic picture of fibrosis. The epididymis in part showed changes such as are often found in retention of the testicle. There was severe old and recent peri-orchitis.

[After this paper was written another specimen attracted my attention. In the tunica vaginalis of a 50 year old man who had died of tuberculosis and diabetes was found a dumbbell-shaped concretion, measuring about 3 by 2 by 2 mm. Chemical examination showed calcium phosphate. Histologically, the outer layer presented the same picture as that of the other specimen. In the center, however, partly calcified fat tissue was seen. As figure 2 shows, part of the fat cells had a thin calcific shell or lining, and many of them contained an irregularly round laminated microconcretion.

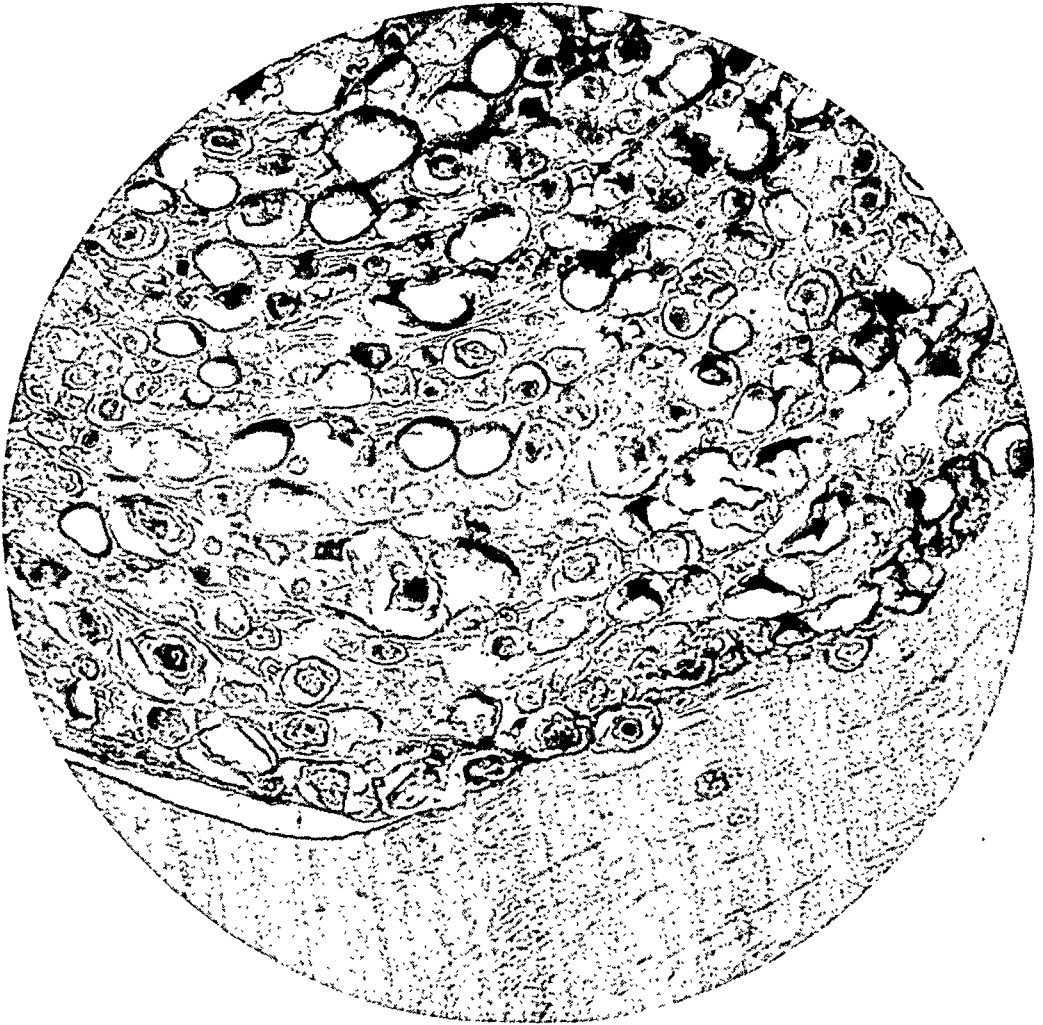


Fig. 2.—Center of a free body, showing fat cells, some of which have thin calcific shells. Many of the fat cells contain each an irregularly round laminated microconcretion.

If continued attention were given to this matter it probably would bring to light a variety of changes in the fat tissue originally forming the nucleus of a free body.]

COMMENT

Most of the free bodies that have been found in the tunica vaginalis have been of small size, ranging from that of a body just macroscopically recognizable to that of a body as large as a pea. Free bodies of

the latter size represent the most common variety, and they may be multiple. Indeed, Sultan³ found from 1,000 to 1,200 free round bodies, varying in size from that of a buckshot to that of a coffee bean, filling the tunica vaginalis in a case of incarcerated congenital hernia. This is a rare exception, however, from 2 to 5 being the average number of bodies of this size (Ritter).

Free bodies larger than these are much rarer. Oberndorfer⁴ stated that they may reach the size of a "plum" and reported one measuring 2 by 2 by 0.5 cm., found in a hydrocele. It was porcelain white and of a cartilaginous consistence, and was made up of concentric layers of anuclear hyaline "connective tissue." A photograph of his specimen shows a central cavity instead of a nucleus, suggesting the possibility that the latter may have been lost in handling. Others have reported such large single free bodies (Chassaignac,^{5a} one 2 by 1.2 cm.; Glass,^{5b} one 1.4 cm. in diameter; Ritter,² one 1.2 by 0.8 cm.; Lavenant,⁷ one 1.3 by 0.6 cm.). Apparently, therefore, the specimen I have described is of unusual size, its measurements definitely exceeding those just mentioned.

Various suggestions have been propounded as to the origin of free bodies of the tunica vaginalis. They may have arisen from: (a) broken-off bits of tissue in periorchitis proliferans (Virchow) or periorchitis villosa (Klebs); (b) detached hydatid bodies of Morgagni (Volkmann; Meyer⁸); (c) incrustated epithelial scales (Luschka; Vauthier); (d) blood coagulums and fibrin clumps after inflammation (Roux).

Although in most of these theories it has been assumed that a detached piece of tissue serves as the nucleus for the formation of a free body, I have not been able in a reasonably exhaustive review of the literature to find a single report in which the statement is made that a nucleus of histologically recognizable fat or of any other tissue has been found in a free body of the tunica vaginalis. Ritter,² Langhans^{5a} and Hartmann^{5a} each mentioned the finding of fat droplets and cholesterol crystals in the center of the free bodies which they studied, but none reported finding fat tissue. However, identifiable pieces of tissue have been described as composing the nuclei of free bodies in other serous cavities.

3. Sultan, D.: *Virchows Arch. f. path. Anat.* **140**:449, 1895.

4. Oberndorfer, S., in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1931, vol. 6, pt. 3, p. 736.

5. (a) Cited by Ritter²; (b) cited by Lavenant.⁷

6. Glass, E.: *Zentralbl. f. Chir.* **47**:266, 1920.

7. Lavenant, M.: *Bull. et mém. Soc. de chir. de Paris* **20**:819, 1928.

8. Meyer, A. W.: *Am. J. Path.* **4**:445, 1928.

Owing to the fact that in the case reported here there was an associated congenital hernia, with an internal ring that was estimated by the surgeon as having had a diameter of approximately 2.5 cm., the question of an abdominal free body was thought to deserve consideration. It is theoretically possible that the free body might have originally been formed in the peritoneal cavity and have lodged secondarily in the tunica vaginalis.

Indeed, Hoche⁹ in an excellent review of the subject of abdominal free bodies reported the finding of a large one, 5.2 by 4.5 cm., which on section showed a gross and histologic picture almost identical with that described here. In the same necropsy were found three lobules of fat tissue, 1.5 by 1 cm. each, attached to the greater omentum by pedicles and possessing on their outer surfaces lamellae of fibrin showing "fibroid changes." In his discussion of the earlier literature Hoche cited several instances of similar bodies being found in various locations in the peritoneal cavity. Three of these are of interest because of their relation to hernial sacs.

In 1850 Canton¹⁰ described a free body, 5 by 3.7 cm., which showed grossly a fatty nucleus the size of a marble, with an outer shell of concentric thin lamellae about 1 cm. thick. No microscopic examination was reported.

Shaw¹⁰ described a free body which was extracted from a hernial sac "after passage from the peritoneal cavity." It was 3.7 by 3 cm. in size, had a central chalky and fatty nucleus and a shell of "fibro-cartilage." Its origin was claimed to be from a detached epiploic appendix. It had no pedicle. No histologic report was mentioned.

Wood¹⁰ reported a free body in a hernial sac, which body in the living patient could be pushed up into the abdominal cavity.

Riedel¹¹ also presented evidence tending to demonstrate the possible origin of such free bodies from fatty appendages of the large bowel. In this connection it is also interesting to note the experimental production of fat-containing abdominal free bodies by Tomellini,¹² who could cause a free body to develop by tying off an epiploic appendix at its base, if, by means of mild chronic inflammation, he made resorption difficult.

It seems highly plausible, therefore, in the absence of other possible sources for the fat tissue which made up the center of the present specimen, that it may have had its origin in either a detached piece of omental tissue or a broken-off epiploic appendix. The possibility of an intraperitoneal origin must be considered in view of the type of hernia presented and because of the evidence afforded by the literature just cited.

9. Hoche, L.: *Arch. de méd. expér. et d'anat. path.* **22**:507, 1910.

10. Cited by Hoche.⁹

11. Riedel: *München. med. Wchnschr.* **52**:2308, 1905.

12. Tomellini, cited by Morpurgo: *Ergebn. d. allg. Path. u. path. Anat.* **12**: 252, 1908.

SUMMARY

In the central cavity of an unusually large free body in the tunica vaginalis testis a piece of fat tissue was found. This fat tissue probably came from an epiploic appendix or from a detached piece of omentum.

In most of the instances of free body in the tunica vaginalis the free body has been of small size. Often there have been multiple free bodies. Theories as to their origin usually ascribe their formation to causes within the tunical sac, as there is usually no communication between the sac and the general peritoneal cavity.

General Reviews

ELASTIC TISSUE

GEORGE M. HASS, M.D.*

BOSTON

INTRODUCTION

A large part of the human body is composed of tissues which are designed in such a way as to enable them to perform mechanical functions. Some of these tissues may be considered as essentially in an intercellular position. They are usually present in the form of fibrils, membranes or matrices. They are fundamentally organic in composition, although inorganic materials may occur in quantities that are sufficient to obscure the basic structure.

The mechanisms which are operative in the formation, maintenance and disintegration of these tissues are not well understood. The tissues are subject to a wide variety of transformations under the influence of many normal and pathologic stimuli of a local or a general nature. By reason of this behavior they are entitled to an important place in many studies of growth, differentiation, senescence, injury and repair.

The intercellular substance with which this article is concerned is known as elastic tissue. It is selected first for presentation because it has been investigated rather completely from several points of view.

HISTORICAL SUMMARY

The ancients must have been familiar with the elastic qualities of various tissues. Yet, Hippocrates makes no reference to this important property. The extensive, though often erroneous, observations on the pulse and blood vessels made by Galen failed to reveal the importance of the elastic nature of the arterial wall. In the "De pulsibus" he noted degrees of softness or hardness of the vascular walls, which he described as fleshlike or as dry and hard, like leather. Hieronymus Fabricius, one of the greatest of the early anatomists, must not have been aware of the true character of the arteries, or else his pupil, William Harvey,

From the Department of Pathology, Harvard Medical School.

* Past Member of the Society of Fellows, Harvard University.

failed to listen carefully to his teachings. Harvey came nearest to a conception of the elasticity of blood vessels when he wrote, "Hence, since Nature makes nothing in vain and does the best everywhere, the nearer arteries are to the heart, the more do they differ from veins in structure. Here, they are stronger and more ligamentous."

In the middle of the eighteenth century, Albrecht von Haller, a remarkable scholar and scientist, made the first careful studies and comments on the elastic property of the connective tissues, designated by him as the *tela cellulosa*. His capacity for perception of basic principles is not more amply demonstrated than in the following excerpts from his dissertation on the *tela cellulosa*: "The primary simple fibre such as we rather comprehend from reason than sense is composed of earthly particles, adhering longitudinally and connected by intervening and cohesive gluten." Then, after a description of the distribution and nature of the fibers, he returned to a consideration of the interfibrillar substance, gluten: "But here the order of nature seems to be that the fibres above mentioned are all originally formed of this gluten." And in a later paragraph he continued: "It seems then, that an albuminous fluid, with a small portion of earth first concretes into filaments, from some pressure whose causes we now pass over." In regard to the elasticity of the *tela cellulosa*, he stated: "It possesses a contractile power different from irritability, which though not demonstrable by experiments, disposes the cellular fibre to shorten itself, though for the most part slowly, after having been stretched." This was a close approximation to the truth, but probably the activity of smooth muscle offered a partial explanation for some of the contractile phenomena which he cited.

Although Haller attributed the elasticity of arteries to the circular fibers, Flemming, his contemporary, more closely approached an accurate understanding of the arterial wall when he wrote: "But when the arteries are fully distended, as the impetus ceases, the distending fluid being no more supplied, they will be left to contract themselves which they do either by the muscular action of the circular fibres or by their natural elasticity or both (but I believe much more by the latter than the former) and so . . ."

The segregation of elastic tissue, collagenous connective tissue and smooth muscle as distinct structural elements awaited the rise of the school of histologists. The founder of histology was Jakob Henle, and to him are attributed the first comprehensive studies on elastic tissue, although Eulenberg and Schwann had made certain minor investigations. Henle (1841) was the first to identify and specify the *elastica* in the walls of blood vessels. Kölliker (1850; 1861) and Donders were also among the early investigators who sought to throw light on the origin, nature and distribution of elastic tissue. The accumulated observations

as recorded by Kölliker (1850) disclose the eager progress made by the histologists and embryologists. The rising school of pathologists soon began to add contributions dealing with the nature of the elastica in disease. Unna perfected a stain by which the alterations in the character of elastic tissue became more apparent. "Elacin," "elastin," "collacin" and "collastin" became common terms of usage. Strenuous debates arose concerning the origin, deterioration and regeneration of the tissue.

The morphologist, proud of his rapid strides but seeking further aid, introduced the tissue to his colleagues. The physiologist failed to be impressed and never became seriously interested in its functional significance. The chemist added his bit in the form of a few analyses, made principally on the normal bovine ligamentum nuchae. Within recent years there has been greater interest, and as a result the physicist and the chemist have advanced experimental evidence, as well as important theoretic considerations, destined to reveal the molecular structure and arrangement of the elastica. Thus, investigators of this tissue stand, at present, with incompleting tasks in all fields of endeavor, especially in those labors necessary to unify the whole—studies of the function and of the formation and maintenance of the elastica in normal and in pathologic states.

ANATOMIC DISTRIBUTION

Elastic tissue is always in association with collagenous tissue. The proportions of each vary within wide ranges. In general, elastica is less abundant in those places where the connective tissue shows loose texture. Numerous elastic fibrils are found in certain cartilages which are noted for their flexibility and elasticity. Rare fibers may be demonstrated in fibrocartilage. In bone there are a few thick elastic fibers, which usually may be traced into the periosteum. Adipose tissue for the most part has only a scant supply of elastic networks. Above all, the intimate association of elastic tissue with smooth muscle is notable. This is so constant that some authors consider the combination as one tissue—a myoelastic tissue. Here, the anastomosing networks pass between and embrace smooth muscle fibers. In voluntary muscle they form a part of the sheaths and extend between the muscle fibers. Their abundance varies, but they are numerous in the ocular muscles and those muscles which are attached to soft parts, such as those of the tongue and face. Cardiac muscle contains but few fibers.

In addition to the foregoing introduction to the general distribution of elastic tissue, it seems necessary to describe in brief detail the arrangement of elastica in the various bodily systems in order that a more complete understanding of the significance of the tissue in normal and in

pathologic processes may be reached. For much of this description, I am indebted to Maximow and Bloom.

Those systems which are designed chiefly for the mechanical transportation of materials from one part of the body to another are supplied with an abundance of elastic tissue. Among these, the cardiovascular and lymphatic systems are of primary importance. The cardiovascular system may be divided for discussion into the heart, the arteries and the veins, while the lymphatic system may be divided into the spleen, the lymph nodes and the lymphatic channels.

The heart does not have an abundant supply of elastic tissue. The elastica is most prominent in the endocardium, especially in that of the left atrium. From here the elastic networks if traced between the muscular crossbars of the atria are found to be in continuity with the elastica of the epicardium. Although there are fibrils in the endocardium and in the pericardium of the ventricles, they seem to be absent from the myocardium except in the adventitia of large blood vessels. In the annuli fibrosi there are few fibers. The atrioventricular valves contain more elastica in their atrial than in their ventricular halves. The leaflets of the aortic and pulmonary valves have numerous delicate fibrils which extend from the corpora arantii to the annuli, as well as other networks, which are especially numerous beneath the endothelium of the ventricular aspect of each leaflet.

The arteries may be divided into the elastic type, the muscular type, the arterioles and the precapillary arterioles.

The arterioles have a thin internal elastic membrane composed of a network of delicate fibrils. Although there are a few fibers in the adventitia, there is no external elastic membrane.

As the arterioles diminish in caliber to about 62 microns the internal elastic membrane disappears and the adventitia soon loses its elastic network, so that the precapillary arterioles, as a rule, are devoid of elastica.

Arteries of the muscular type have an internal elastic membrane, which in the smaller vessels is an elastic network. In the larger arteries there are subendothelial fibers, and the internal elastic membrane assumes a platelike, fenestrated appearance. This internal elastic membrane may be split into two or three layers, especially where these arteries branch. The increase in the size of these vessels is accompanied by an increase in the number of elastic fibers, which embrace the smooth muscle cells of the media. In the intermediate muscle layers, the elastica may form fenestrated concentric bands, which alternate with bands of muscle fibers. This medial network, which is continuous with that of the intima, fuses externally with the elastica externa. From this condensed layer, fibrils pass outward to enmesh the tissue elements of the adventitia.

Large arteries of the transitional musculoelastic or elastic type have a variable distribution of elastica. As a rule, there are subendothelial fibers which unite with a thick fenestrated internal elastic membrane that in adult life may be composed of several lamellae. The media of these arteries is composed largely of elastic tissue. In the aorta this consists of from fifty to sixty-five concentrically laminated thick elastic membranes. These are joined by delicate elastic fibers and are separated for the most part by collagenous connective tissue and smooth muscle fibers. There is no elastica externa, but in the outer layer of the media are found abundant networks, which anastomose with the rich supply of elastic tissue in the adventitia.

Many variations from the model arteries have been described. The media of the visceral arteries may be composed of an internal muscular coat and an external elastic layer. The cerebral arteries have a well developed elastica interna and very little elastica in the media and adventitia. In the renal arteries the networks are unusually prominent. The coronary arteries have internal and external elastic membranes. Although the inner layers of the media of the coronary arteries are rich in elastica, the outer portions are composed principally of smooth muscle. Other variations in arteries, which accompany certain physiologic and pathologic changes, will be discussed elsewhere.

Veins.—Veins may be divided into those of small, medium and large caliber. There are great variations in the structure of veins and in their corresponding content of elastic tissue. They are much less elastic than arteries, and their elastica is less well developed. In veins larger than 200 microns in caliber, elastica may be found. This is composed of delicate networks and is found in the media and adventitia. In the veins of medium caliber (from 2 to 9 mm.) a few inconspicuous elastic fibers may be distinguished in the intima, and a network of thick longitudinal fibers occasionally is found between the intima and the media. This network never assumes the form of a fenestrated membrane. In certain vessels, such as the saphenous vein, the innermost layer of the media is relatively rich in elastica. In larger veins the external layer of the media has flat networks of longitudinal elastic fibers which often separate the circular muscles into layers. These networks are connected with the fairly prominent meshes of adventitial elastica.

The veins of large caliber usually have a few fibrils in the intima and media, while thick prominent fibers are numerous in the connective tissue of the broad adventitia. Smaller fibers are found in the inner muscular layer which lies adjacent to the media or sometimes next to the intima, if the media is absent.

Each valve of a vein contains a rather thick network of fibers on the side which is directed toward the lumen. These fibers are continuous with the intimal elastica.

Lymphatics.—Elastic fibers occur in the walls of all lymphatics which are of sufficient size to have valves and smooth muscle in their walls. In lymphatics with a diameter greater than 2 mm. there are longitudinal interlacing fibers in the intima, delicate intermuscular fibrils in the media and in the adventitia a moderate amount of elastica, which for the most part is disposed tangentially. The thoracic duct has several intimal layers of delicate fibrils which are directed longitudinally. Near the junction of the intima and media an internal elastic membrane is formed by condensation of the fibrillar network. From this structure anastomosing fibers penetrate the media and join with the thick longitudinal fibers of the adventitia.

The lymph nodes are poorly supplied with elastic tissue, but in the capsules and occasionally in the trabeculae delicate fibrils may be found.

The spleen contains an abundance of elastica. It is especially plentiful in the capsule, where many fibrils, as well as elastic membranes, may be found. These are usually most numerous in the inner layers of the capsule, whence they are continued into the trabeculae, where elastica often exceeds collagenous connective tissue in amount. There are rare fibers in the reticulum of the white pulp. These are most numerous around the central arteries and at the periphery of the malpighian bodies. There is no elastica in the red pulp. As soon as arteries pass into the red pulp, they lose their complement of elastic tissue.

Skin.—The elastic tissue of the integument has been studied thoroughly. The hypodermis has but few fibers. In the derma thick networks ramify independently of spaces between the bundles of collagen. They tend to condense around the adnexa. An interesting arrangement is the attachment of arrectores pilorum muscles to networks of elastica. Beneath the epithelium and in the papillae, numerous delicate fibers form a continuous network. The elastica of the cheek differs from that which occurs elsewhere. It is composed of a dense feltwork of closely arranged twisted fibers, which lies just beneath the epithelium. In general, where the skin folds easily, the elastica is scanty. Where the skin is bound closely to the underlying structures, the fibers usually are more thick and numerous.

Respiratory Tract.—Elastic tissue is found throughout the extent of the respiratory tract. Sparsely distributed delicate fibrils are found in the nasal mucosa. In the larynx, the arytenoid cartilages, although hyaline at their bases, have numerous elastic fibers in the matrix of the uppermost portions. The true vocal cords are composed largely of bands

of elastic fibers. In the lamina propria of the trachea many delicate networks are found. The elastic fibers are so closely arranged around the tracheal cartilages that a compact membrane is formed. The smooth muscle fibers which pass between the free ends of the incomplete cartilaginous rings are inserted principally into dense bundles of elastica which surround the trachea and its cartilages. The lamina propria of the bronchi is richly supplied with elastic fibers that are continuous with the abundant fibrillar network which enmeshes the smooth muscle cells of the bronchial walls and extends throughout the enveloping connective tissue, tending to condense around bronchial cartilages. In the respiratory bronchioles (less than 5 mm. in caliber) the myoelastic membrane is very prominent. Many closely arranged straight thin fibrils are found in the walls of the alveolar ducts and around the openings into the alveolar sacs. The interalveolar septums have a compact meshwork of reticular fibers, but the elastic fibers are few. The visceral pleura contains several prominent layers of elastica. These course at various angles to the plane of the surface.

Alimentary Tract.—The alimentary tract, although well supplied with smooth muscle, has much less elastic tissue than the respiratory tract. The mucous membrane of the mouth contains elastic networks which, except for a larger number of delicate fibers, are similar to those in the skin. In the mucous membrane of the cheek, as well as in the derma, there is an unusually abundant supply of elastica. The soft palate has dense networks, which lie between the lamina propria and the mucous glands of the submucosa. However, on the nasal side of the soft palate similar arrangements of elastica separate the mixed glands of the lamina propria from the muscle. No elastic fibers are found in the periodontal membrane. Beneath the epithelium of the tonsils there is a moderate amount of elastica, which is continued into the cores of the tonsillar folds. In place of a muscularis mucosae the pharynx has a thick layer of fibers, which principally are disposed in a longitudinal direction. Where the pharynx merges with the esophagus, the elastica becomes thinner as the networks are succeeded by the muscularis mucosae, which retains a few delicate fibers. In the fornix the layer of elastica blends into the periosteum of the skull. Throughout the lamina propria delicate fibrils are found both in the pharynx and in the esophagus. The latter has, in addition, coarse networks which ramify throughout the submucosa. The stomach has very little elastica in its wall, especially in the lamina propria. The intestine has a few delicate networks which surround the vessels in the lamina propria and accompany the smooth muscle of the muscularis mucosa. Although the networks increase in prominence in the submucosa, the muscular layers contain only a few fibrils.

Urogenital System.—The kidney has no elastica except that which is associated with vessels. It may be noted that the afferent arterioles have a delicate elastica interna, which disappears with the branching of the vessels to form glomerular arterioles. The efferent arterioles have no elastic membrane. The lamina propria of the renal pelvis, ureter and bladder has a few delicate networks, which are continuous with the fairly abundant elastica of the tunica muscularis. The lamina propria of the urethra is rich in elastic networks.

The genital tract of the male contains much elastic tissue, while that of the female is less well supplied. Elastic nets are present in the tunica albuginea, mediastinum and septula of the testis. The singular basement membrane of the seminiferous tubule contains many very delicate fibrils. Elastica is prominent in the corpora cavernosa, not only between the cavernous blood channels but also in the tunica albuginea. The ovary has no elastica in the cortex, but there are numerous networks in the medulla. The fallopian tube has a few fibers in the muscularis. In the uterus there is no elastica in the endometrium or in the subjacent muscle. Fibers are present in large numbers in the external portions of the uterine musculature, in the cervix and in the vaginal wall. The lobule of the breast normally has no elastica. In the lactating gland elastic fibrils may be found, especially around the excretory ducts.

Biliary and Pancreatic Ducts.—The biliary system has a small amount of elastica around the intrahepatic ducts. The tissue increases in rough proportion to increase in caliber of the ducts. The largest number of fibers are found in the walls of the extrahepatic ducts and in those of the gallbladder. In the latter they tend to accompany the smooth muscle bundles, only a few fibrils being found elsewhere.

The pancreas has only a few delicate networks. These are located around ducts.

Osseous System.—The periosteum contains a few networks, which are more prominent in the external layer. From this layer thick fibers occasionally may be traced into the cortical bone. A few fibers are present in the intervertebral disks and in the capsules of joints.

Central Nervous System.—Except that present in blood vessels, the only elastica in the central nervous system is confined principally to a few networks in the dura and leptomeninges.

Eye.—The eye has a plentiful supply of elastica. The delicate networks of the sclera and those of the choroid join to form the rich meshwork of the lamina suprachoroidea and lamina cribrosa. In the substantia propria of the cornea a series of very delicate networks are found. These are more easily found anterior to Descemet's membrane. Although this membrane resembles elastica in staining qualities, the reactions are not typical. Beneath the pigment epithelium there is a narrow zone of

delicate fibrils. There are numerous fibrils in the ciliary body, but none is found in the uvea. The eyelids have abundant networks.

Ear.—The auricle of the ear has an irregular plate of elastic cartilage, about which there are dense elastic networks. Although the tympanum is composed principally of collagen, delicate elastic fibrils are present, most prominent in the central zone. The eustachian tube is enclosed partially by elastic cartilage.

EMBRYOLOGY

It would be impossible to discuss the complete embryologic evolution and development of the elastica in this limited space. Therefore, it seems appropriate to dwell briefly on the tinctorial qualities of the elastica as it matures, the time and site of its first appearance in certain vertebrates and its distribution in developing chick and human embryos.

The vascular system is the first bodily part to be supplied with elastic tissue. The larvae of certain Amphibia, namely, the salamander (Flemming) and the axolotl (Spalteholz), have been studied. In the former, when it is 3 to 4 cm. in length, elastic fibers appear in the *Mesenterialwurzel*. In the latter, when it is 9 to 10 mm. in length, they may be found in the truncus arteriosus. Among the Aves, Spalteholz demonstrated elastica in the three day chick embryo and the four day duck embryo. In the human embryo elastica first makes its appearance during the third or fourth week, at which time it is found in the aorta (Röthig). The development of the elastica in the chick embryo was studied by Nakai. During the fifth day, two days after the heart begins to beat, fibrils may be found at the base of the aorta and the pulmonary artery, in the peripheral layer of each vessel. By the ninth day, elastica is demonstrable in the epicardium, and about the tenth day it appears in the tissues around joints and insertions of the extremities. By the fourteenth day it is distributed throughout the interstitial tissues but does not appear in the organs until later.

The study of human embryos has been concerned chiefly with the vascular system, lungs and skin. Röthig demonstrated elastica in the aorta during the third or fourth week. Hewer found that fibrils in the cerebral and coronary arteries were well developed as early as the sixteenth week. However, even at the time of gestation certain vessels, notably those of the adrenal, pituitary and thymus, and veins of the renal cortex were supplied with very little elastica. Linser found that the networks in the pulmonary vessels were demonstrable as early as the tenth week but that they did not gain their full fetal complement until the middle of the fifth month. This distribution is changed after birth. Within a period of two or three months of extrauterine life the veins show a relative increase and the arteries a relative decrease in the amount of elastic tissue—thus reaching a stage comparable to that found

in the adult lung. In the course of the third month of embryonic life a thin refractile membrane appears beneath the endothelium of the brachial artery. This does not stain electively until the fourth month; nevertheless it is the anlage of the mature elastica interna (Maximow). During the fourth month the elastica externa makes its appearance. Coincident with the increase in the number of smooth muscle cells in the media, an increasing number of elastic fibrils develop. Thus, the medium-sized arteries, of which the brachial is an example, attain their complement of networks.

Linser investigated the development of elastica in the lungs of human embryos. Early in the fourth month fibrils appear along the larger bronchi. These increase in number and assume a purposeful laminate arrangement, so that at the beginning of the fifth month there are six to eight delicate bands beneath the epithelium. By this time elastic networks are forming among the smooth muscle bundles as well as in the peribronchial tissues. In the latter situation they tend to condense around the cartilages, while only rare fibrils are found in the cartilages. During the fifth month a few fibrils appear in the lung parenchyma, especially along small vessels and adjacent to epithelial-lined structures. It is not until the seventh month that fibrils are distinguishable in the stroma of the parenchyma. By the end of the seventh month the elastica reaches the full state of antenatal development. During the first month of extra-uterine life the elastica becomes more abundant and reaches the state of differentiation which is found in the adult lung.

The progressive stages of differentiation of the pulmonary elastic tissue are analogous to those which were described by Maximow as occurring in the internal elastic membrane of the brachial artery. The elastic fibrils are at first only weakly stainable by the specific stains. The intensity of the staining reaction increases gradually, but with unusual rapidity during the sixth month. But, surprising though it may seem, the full depth of staining, which corresponds to that of the vascular elastica, is not attained until after several days of extrauterine life. The observations of Sudsuki and Röthig in general were in agreement with those which have been described.

Linser found that the development of the elastica in the lungs of a number of mammals was similar to that which occurred in man. The chief difference was in the rapidity of formation. As a general rule, he found that the more active the animal the more rapid were the formation and maturation of elastic tissue.

The development of elastic tissue in the skin has been studied in detail by Lynch. It appears in the blood vessels at the fifth month and in the corium during the sixth month. According to White, there is a great increase in the amount during the eighth month. Hewer found an abundance of elastica in the skin at the fourth month. It may be

that certain discrepancies are explicable on the basis that there is a great variation in the amount of elastica from place to place.

Hewer attempted to correlate the time of appearance of elastica in the human embryo with the beginning of functional activity of tissues and organs. In the study the basement membranes apparently were considered as a part of the elastic tissue system. The first appearance of elastica in the kidneys was in the interstitial tissue at eight weeks. At sixteen weeks there was a definite basement membrane to Bowman's capsule, the convoluted tubules and Henle's loop. Previously, during the sixth to the eighth week, the ureter had gained a basement membrane. The author assumed that the kidney began to function at about the twelfth week. Elastica was found in the spleen as early as the twelfth week and was present in abundance in the skin by the sixteenth week. In contrast to the vascular system, lungs and skin, the alimentary tract showed a scanty and much retarded development of elastica. A basement membrane appeared beneath the epithelium of the esophagus during the twelfth week. There was no elastica in the small intestine. A few fibrils were found in the muscularis mucosae of the stomach, cecum and appendix after the sixteenth week. The parathyroid contained no fibrils. By the sixteenth week the thyroid follicles had been supplied with a basement membrane and the glandular stroma showed numerous fibrils. The elastica began to develop in the stroma of the adrenals at eight weeks and in the pituitary at thirty-two weeks. The tunica albuginea, trabeculae and basement membranes of the testis showed some elastica at eighteen weeks. As early as the sixth week fibrils began to appear beneath the germinal epithelium of the ovary, and a few fibers penetrated the stroma. No elastica was found in the thymus. By the sixteenth week there was an "elastic" basement membrane of the choroid plexus. At this time there were a few delicate fibrils in the pia-arachnoid. After this thorough study, Hewer was unable to arrive at definite conclusions concerning the influence of function on the origin and maturation of the elastica. The chief difficulty was the lack of knowledge concerning the time of onset of function in most of the organs and tissues.

PHYLOGENY

Exact information concerning the occurrence and distribution of elastic tissue in the invertebrates and lower vertebrates is meager. From the accumulated data, one might believe that what occurred phylogenetically in the animal series is analogous to that which occurs ontogenetically in man.

In the invertebrate series, studies have been made on certain Mollusca and Chordata. Argaud (1909) stated that there were no true elastic fibers in Mollusca, but there were fibrils which had the physical character-

istics without the staining qualities of elastica. In Arion, Argaud (1909) found no fibrils of this nature. In *Eledone moschata*, beneath vascular endothelium there was a hyaline limiting membrane which had the optical but not the staining reactions of elastic tissue. Inasmuch as A. Aschoff (1893) stated that the first anlage of the elastica interna in the human embryo appeared as a hyaline membrane, Argaud raised the question as to whether this hyaline membrane could be connected phylogenetically with the hyaline membrane of Mollusca. Schiefferdecker studied *Sepia officinalis* but could find no elastic fibers stainable as such. Wetkamp found in the typhlosole and in the stomach wall of *Anodonta cellensis* not only connective tissue but also elastic fibrils. Indeed, the whole intestine seemed to be enclosed in an elastic net. Schiefferdecker was unable to demonstrate elastic fibrils in the typhlosole of *Anodonta* and *Unio*. Among Chordata, Bütschli studied the amphioxus and found elastic fibrils in the perichordal tissues. Schiefferdecker could not confirm this.

Observations relative to the vertebrate series were less contradictory. Two Cyclostomata, *Petromyzon marinus* and *Petromyzon fluviatilis*, were studied. Argaud (1908) found no elastica in the former. In the latter, Schiefferdecker noted around the chorda a membrane which resembled elastic tissue. In two Ganoidei, *Acipenser sturio* and *Acipenser ruthenus*, there were well formed typical elastic fibers, especially in the walls of blood vessels, around the chorda and along the vertebral column (Schiefferdecker). In two Selachii, *Scyllium stellare* and *Torpedo ocellata*, Schiefferdecker found an abundance of elastica through the body. The same has been found to be true for all higher vertebrates. The amount and distribution of elastica differ widely, however, as may be inferred from the careful descriptions of the pulmonary elastica of various Amphibia, Reptilia, Aves and Mammalia (Ogawa).

From the accumulated observations it seems that in the lower animals there is a tissue which has the physical properties but not the staining reactions of elastica. Higher in the animal scale, in *Petromyzon*, elastica tissue which can be differentiated by staining reactions first appears. Even in this instance, the staining qualities are not quite typical. In Ganoidei characteristic elastica makes its appearance. Phylogenetically, the early appearance of elastica and the assumption of the differential staining reaction are comparable to those developmental stages of the elastica in man. In the latter instance, first the fiber is refractory to the stain, secondly the fiber is impregnated weakly, and finally the fiber assumes full differential staining qualities. In this regard it is of interest that a similar development of morphologic and tinctorial properties was described by Bloom, who studied the formation of elastic fibers in tissue culture.

Finally, the reversal of the developmental sequence occurs in certain regressive changes which will be considered in the discussion of the pathology of the elastica.

HISTOGENESIS

Controversial observations and spirited polemics have marked the progress of knowledge of the mode and site of origin of the elastic elements of the connective tissues and cartilage.

Before the numerous theories are discussed certain fundamental questions which bear on all hypotheses must be considered. First, does the elastic fibril originate by coalescence of granules or is it developed as a continuous delicate fibril? Second, if it develops as a fiber, is it from the beginning a fully differentiated fiber, or is it formed, by means which for the present one may disregard, from a preelastic fibril, from an undifferentiated fiber or from a collagen fiber?

The consensus is that the elastic fiber, whether it is in cartilage or elsewhere, first appears as a continuous fibril. Evidence to the contrary has been advanced by Ranvier, Gerlach, Deutschmann, Loisel, Gardner and de Kervily. Ranvier held that the fibers were formed by a fusion of granules. Gerlach and Deutschmann demonstrated granules, which were bound alongside cells. They contended that these were the precursors of the elastic fibril. Loisel believed that the elastica arose as a fiber derived from the cell protoplasm, but in the intercellular matrix he noted elastic granules the purpose of which seemed to be to augment the size of the formed fiber. Gardner found in the protoplasm of cells tiny granules, which became aligned so as to form intracytoplasmic fibrils by coalescence. De Kervily showed by silver stains that a large number of fibrils, especially in the cartilages and perichondrium of the respiratory tract, appeared to be composed of rows of small granules.

Other authors were content to consider the original structure as a fibril, but they failed to agree as to the exact nature of the fiber. Loisel, Linser and von Korff contended that the product of the fibroblast was an undifferentiated fiber which could be transformed into either collagenous or elastic elements. Virchow (1851), Schiffmann, Gugot, Fuss and Krösing (see Röthig) believed that elastic fibers arose by alteration of the character of the collagenous fibril. Other authors expressed the opinion that the elastic elements were formed independently and from the beginning were destined to be elastic tissue.

Further questions which faced the investigators may be outlined as follows: Does the elastic fiber originate from cell protoplasm or from the intercellular matrix? In either instance, is the formation dependent on specific cellular activity? If cellular activity is of fundamental importance, is there a specific cell whose sole function is the elaboration of the elastic fibril? What is the mechanism by which the fiber is controlled

after it has gained an intercellular position? What part do mechanical or other forces play in this mechanism?

For purposes of correlation one may take the liberty of classifying the results of many studies into three groups. There were those authors who believed in the cellular origin of the elastica. Their contemporaries advanced a contradictory hypothesis which concerned itself with the formation of fibrils in the intercellular substance without specific participation of cells. The third group of observers combined the afore-said cellular and intercellular theories.

The cellular theory of origin includes all points of view which are concerned with fibril formation by virtue of cellular activity from nuclear material, intracytoplasmic granules, cytoplasm, ectoplasm or secreted substances. Advocates of this theory were Schwann, Henle (1841), Boll, Hertwig, Gerlach, Fol, Taddei, Nakai, Spalteholz, Jores (1907), Ladwig, de Kervily, Orsos (1926) and Krompecher (1928).

The second, or intercellular, theory was supported by Kölliker (1850), Henle (1852), Schwalbe, Weismann, Rabl-Rückhard, Kollmann and Fuss.

The third theory was developed on the basis of a new conception of the fundamental nature of the intercellular ground substance. Hansen, Spüler, Loisel, Mall, Flemming, Geipel, Meves, Hueck and Ladwig supported this hypothesis.

Even within the separate groups there were variations of opinion. For this reason it seemed worth while to present briefly the ideas of certain authors.

Henle in his earlier writings regarded elastic fibers as originating from the nuclei of connective tissue cells, although subsequently he was inclined toward the point of view that they arose in the intercellular substance.

Hertwig found that the fibers first appeared along the margins of cells. He believed that they were constructed from the protoplasm by specific activity of the cells from which they arose.

Gerlach contended that the fibers, at first very delicate and difficult to distinguish, were formed at the cell surfaces and later were separated from the cell.

Fol proposed the theory that elastic elements in cartilages were never a differentiation product of the cytoplasm but assumed fibrillar form in the coagulum of the cell secretion.

Taddei agreed with the conception that young elastic fibers were differentiated in the protoplasm of connective tissue cells and eventually were cast off into the intercellular spaces.

Nakai was able to follow the stages by which the processes of mesenchymal cells were converted into elastic fibrils.

Jores (1907) supported the contention that elastic fibers developed from cell protoplasm without passing through an intermediate connective tissue fiber stage. He arrived at these conclusions after a series of careful studies.

Ladwig agreed with those who believed that elastic fibers were differentiated along the periphery of fibroblasts.

Spalteholz believed that his studies favored the intracellular theory of origin.

De Kervily demonstrated in certain cartilages of human embryos fusiform cells which contained protoplasmic granules that had an affinity for silver. He believed that these granules coalesced and in combination with the cell protoplasm gave rise to the elastic fibril. Also, he demonstrated intracellular granules which stained electively with elastic tissue stains. He assumed that these were the basic substances of the elastic fibril.

Orsos (1926) found in the same cell two types of fibrils, having different tinctorial and optical properties. The staining reactions indicated that one type was composed of an albuminoid substance and the other of globulin. The former gave rise to collagen fibrils, and the latter became differentiated into the "elastica."

Loisel and Krompecher were not content to accept the majority opinion that the fibroblast or undifferentiated connective tissue cell elaborated the elastic fibril. They presented evidence in support of a theory that the fibril formation was restricted to a specific type of mesenchymal cell, which had no power to form other types of fibers. Loisel designated this cell as an "elastoblast." Both observers believed that this cell could be recognized by its distinctive histologic structure. Krompecher believed that in pathologic as well as in physiologic states the elastic elements were formed along the borders of the elastoblasts and that as soon as the elastic elements were differentiated the specific cells tended to disappear and no new fibers developed unless there was a regrowth of elastoblasts.

The few authors who believed in the origin of the elastic fibril solely from the intercellular matrix did not undertake the complete studies that were made by those who advocated participation of the cell in the formation.

Kölliker (1850) and Schwalbe agreed that the elastica was formed by a peculiar transformation of the intercellular substance of the connective tissue anlage. They believed that all large fibers developed by an increase in size of the small delicate fibrils.

Rabl-Rückhard contended that the elastic fibrils in the cartilage of the ear were formed not from the cells but by differentiation of a part of the hyaline ground substance.

Weismann and Kollmann believed that formative powers were present in the intercellular substance, by which the fibrils were developed.

Matsukoa attributed the new formation of elastic tissue in regenerating cartilage to a morphologic and chemical change of the intercellular substance.

Fuss stated that the cells had no direct part in the formation of fibrils but that these arose from fibers which were chemically identical with collagenous fibers.

The blending of divergent opinions found expression in the conclusions of several authors who gave due consideration to the part played not only by the cell but also by the intercellular materials.

Spüler expressed the opinion that elastic elements were formed by the cells. He believed, however, that the formation was not necessarily dependent on the cell body but that the development of the fibril could occur at a distance from the cell in the ground substance. In 1897 he stated that the outer zones of the cell were a part of the ground substance. These zones seemed to have an inherent formative ability and could readily be split off from the cell.

Loisel believed that certain specific cells, the elastoblasts, formed fibrils at the expense of their processes and their protoplasmic periphery. These fibrils became isolated in the form of a protoplasmic spindle enclosed by a fibrillar mantle. After separation from the cell most of the fibers assumed the character of elastic fibers, and the remainder became a part of the connective tissue. Furthermore, in the isolated protoplasmic part, elastic granules appeared. Their purpose seemed to be for the augmentation of the elastic fibril. Later, the growth of the fiber seemed to occur through a transformation of connective tissue substance into elastic substance.

Hansen agreed with the belief that there was a transformation of protoplasm and protoplasmic processes into elastic fibrils, but he concluded that in cartilage the fibrils were developed from the intercellular matrix.

Mall divided the protoplasm of the mesenchymal syncytium into endoplasm and ectoplasm. He believed that elastic and collagenous fibers arose in the ectoplasm and that one cell was concerned in the formation of both tissues. Flemming also believed that the fibrils took form in the ectoplasmic mantle of cells. He contended that this mode of origin would explain the apparent development of fibers in the intercellular matrix of cartilage.

Geipel concluded that the elastica was formed in the cell sheath or mantle and that it was a matter of individual measurement whether one might consider the fibers as a constituent part of the cell or as a part of the earliest deposit of the intercellular substance.

Meves maintained that the intercellular substance had an inherent ability to produce new fibrils.

Hueck supported a belief which is concerned with a ground substance formed by a thickening of the protoplasmic margins and a continuous recurring separation of these parts from the protoplasm so as to form an undifferentiated intercellular substance from which elastic and collagenous fibrils may arise.

Ladwig on the basis of heterotransplantation experiments concluded that elastic fibrils differentiated along the margins of fibroblasts.

Bloom found that the elastic fibers which developed in tissue cultures of guinea pig heart muscle were always extracellular in position.

It seems logical at this point to consider briefly a few factors which may exert an influence on the origin and augmentation of the elastic fibril. These may be evolutionary, physical, chemical, hereditary or hormonal. The hereditary, the hormonal and to a certain extent the chemical factors will be discussed in the part of this review which deals with the pathology of the elastica. The phylogenesis of elastic tissue and its recapitulation in the embryologic development of the human embryo have been discussed under the appropriate headings.

Physical factors of stress and strain were believed by His to be of great importance in the development of connective tissue structures. Roux, a leader of the "mechanistic school," contended at one time that function and action were the sole influences which initiated and guided the development of the connective tissue structures. Later, he admitted that this idea failed to explain the intricate anatomic character of certain connective tissue elements. He classified these structures as nonfunctional and hereditary. Despite the difficulties of actual proof, he contended that there were two great stages of development. The first period included the embryonic state, during which time the constituent parts appeared, differentiated and grew by virtue of inherent qualities. The second period was characterized by a more complete development of the various parts under the guiding influence of stimuli.

Melnikow-Raswedenkow favored the theory that elastic tissue normally made its appearance where there was a mechanical necessity. Jores (1902) gave certain good reasons for his opposition to this point of view. Scagliosi in instances of phlebectasia described new formation of elastic tissue in those sites where the wall of the blood vessel had been weakened by medial degeneration. Maximow and Bloom concluded that in general the amount and degree of development of the elastica in the wall of the blood vessel were more or less proportional to the pressure of blood within the vessel. Bloom found that elastic fibers were formed in tissue cultures of embryonic guinea pig heart muscle. He stated that although contractile pulsation of the

muscle explant was not essential for their formation, they attained their greatest development under its influence. Thus, what investigators have learned of the phylogenesis, embryonic development, normal anatomic distribution and in vitro cultivation of elastic tissue tends to support the hypothesis that mechanical forces may exert an influence on the genesis and development of the elastica, especially if those forces are rhythmic and fluctuating.

In the preceding accounts, a discussion of the participation of a theoretical chemical substance, elastin, in the formation and maturation of elastica has been omitted. Ranke (see Hueck) and Hueck were two of the several authors who advocated the hypothesis that the fibers which are formed may be impregnated with either collagen or elastin and that this impregnation will determine the ultimate nature of the fiber.

Bierich believed that certain alterations in the physicochemical condition of the collagen fibers fitted them for impregnation with elastin. The more or less variable and interchangeable staining reactions of collagen and elastin in various pathologic states have induced many authors to accept the stated hypothesis. Unna (1928) has discussed the possible chemical nature of these substances.

Further indication that elastin is a specific chemical substance with which fibers may become impregnated has been advanced by tissue culture studies. Erdmann found that elastic fibrils, as judged by staining reactions, were formed in tissue cultures only in those instances in which elastin was present in the tissue prior to explantation. Bloom found that in cultures of embryonic aorta the elastic fibrils which developed took form near the ends of the explant and seemed to be continuous with the preformed elastica in the media. Odiette agreed with the observations of Erdmann. He was able to influence the in vitro development and degeneration of elastic fibers by using various combinations of amino acids in the culture medium. Therefore it seems, from the accumulated facts, that future investigations will be concerned with a substance or substances which are elaborated from relatively simple chemical compounds and deposited in the elective sites. It may be predicted that such substances will be of such a labile nature as to be resorbed, redeposited and augmented in response to certain physiologic stimuli and as a result of certain pathologic processes.

From the foregoing considerations it may be concluded that:

1. Maturation of the elastic tissue morphologically and tinctorially is a relatively slow process.
2. Ontogenetic development of the individual fibril in man simulates the development which occurs phylogenetically.

3. Fibril formation, if influenced by stimuli in the same way as the collagenous tissue, does not respond in a like manner either as to time, place or degree.

4. Embryonic sequences indicate that although the structure and distribution of elastic tissue may in part be predetermined, functional demands, beginning in early embryonic life, also play an important role.

5. The sequences in the development of elastic fiber networks, if one adheres to a cellular theory of origin, remain obscure even though one accepts the postulate that there is a continuous change of endoplasm into ectoplasm.

PROPERTIES

The physical and chemical properties of elastic tissue differ from those of collagenous connective tissue. In treating of this subject the terms "elastin" and "collagen" will be used frequently. These terms are merely names given to the material which composes each type of tissue.

The exact role of each tissue in response to the application of force is unknown. Certain inferences have been made and handed on from one generation to another without proper justification. Within certain limits, after deformation these tissues promptly resume their original form. Inasmuch as one type of tissue is usually accompanied by the other in varied proportion, arrangement and compactness, the part played by each tissue or other elements with which they commonly are associated in the resumption of form has not been studied adequately. Be that as it may, certain observations have led most authors to believe that the elastic tissue has, in the common understanding of the term, great elasticity, while collagen has little inherent power of resumption of form after great deformation. Although knowledge is meager, by an analysis of the physical and chemical nature of elastic tissue certain pertinent questions may be approached. First, how is the tissue designed? In answering this question, the anatomic distribution throughout the body, the microscopic structure of the constituent parts and the more minute physical structure deserve consideration. Secondly, is this design of such nature that it fulfils the requirements by which materials become possessed of great elasticity? In this regard certain laws of elasticity, both physical as applied to rods and wires and physicochemical as applied to elastic colloids, will be considered.

Physical Properties.—The elastica may occur in the form of circular fibrils, as flat bands or as membranes which often are fenestrated. The tissue is yellow. As a rule, it is composed of fibrils which form continuous networks. Normally, no free ends can be found. The unit fibers, band or membranes vary in diameter from almost ultramicro-

scopic dimensions to between 10 and 12 microns. When they are under normal tension, they tend to be straight. If this tension is reduced, they assume an undulate, spiral or angulate form. If a greater than normal force is applied, they exhibit extraordinary extensibility, and as soon as the deforming force is removed, they have an inherent capacity for returning quickly to almost their original shape. At the limit of extensibility they usually rupture in a transverse direction, without splitting, and the broken ends tend to curl in a spiral manner. The extensibility is greater in the long axis of the fiber than in a direction perpendicular to the axis (Wohlisch). In their natural state they are highly refractile and almost isotropic. With drying or stretching they become doubly refractile. While they are drying there is a great reduction in thickness but very little proportional diminution in length (Wohlisch). The fibrils are paramagnetic and exhibit electric and magnetic anisotropy. If one assumes that basophilic matter is on the positive pole and acidophilic matter on the negative pole, elastic tissue is positive to collagenous tissue, and a difference in electrical potential must exist between the two.

There is little similarity between the thermoelastic characteristics of this tissue and those of rubber, with which it has been compared so frequently (Wohlisch). As the temperature is increased from 0 to 60 C. the fibrils soften, gradually diminish in length and increase in thickness. Up to 60 C. these changes are reversible. Above 60 C. the fibers become hard, and there is a decrease in volume, which is evidenced by a diminution in length and breadth.

Theoretic conceptions of the fundamental physical structure of the elastic fiber have been advanced on the basis of data obtained by roentgen ray spectrographic analysis by Herzog and Gonell. These observers concluded that collagenous and elastic fibrils were composed of regularly oriented microcrystals. From their observations, if fiber diagrams of roentgen ray diffraction patterns may be used as a source of authority, it may be contended that the main valence chains of the elastic fiber must extend in the long axis of the fibril, while weak or side valences are assumed vertically. The main valence chains would be grouped to form micellae, which may be considered as the primary units of the fiber. The data which are presented by them are not complete or entirely convincing. Further studies should certainly be made.

Chemical Properties.—The elastic tissue is composed of albuminoids which belong to the group of scleroproteins. In contrast to collagen, it is very resistant to the action of chemical agents (Satterthwaite). It is not soluble in acetic acid, potassium hydroxide or hot water. It is slowly digested by pepsin in an acid solution and by trypsin in an alkaline solution. The ease with which fibers are digested varies accord-

ing to the source from which the fibers are obtained (Marriott). When digested by trypsin in an alkaline medium, the fibers do not become thinner but break up into globules, which are held in position by an external sheath that may represent the substance with which fibers are "coated" or "impregnated." The fibers, as exemplified in certain pathologic states, have an affinity for calcium, iron and silver. Exposure of the skin of mice to roentgen irradiation, tar or arsenic causes an increase in the number of "elastic" fibrils (Bierich, 1922). Later Bierich and Rosenbloom used the term "resorcin fibres" to designate certain fibrils which they produced experimentally by depression of the

TABLE 1.—*Comparison of Collagen and Elastin (After Cohnheim)*

	Collagen	Elastin
Aminoacetic acid.....	19.25	25.75
Alanine.....	3.0	6.53
Valine.....	1.0
Leucine.....	6.75	21.38
Aspartic acid.....	0.56
Glutamic acid.....	14.0	0.76
Proline.....	7.7	1.74
Oxypyrrrolidin carbonic acid.....	6.4
Phenylalanine.....	0.4	3.89
Tyrosine.....	0.34
Histidine.....	0.4	0.53
Lysine.....	5.6	2.48
Arginine.....	9.3	1.86
Ammonia.....	0.43	0.05

TABLE 2.—*Values Obtained by Chittenden and Hart in Analysis of Elastin*

Carbon.....	54.24
Hydrogen.....	7.27
Nitrogen.....	16.70
Sulfur.....	0.30
Oxygen.....	21.79
Ash.....	0.90

p_H of collagen to low values. They considered these to be products of hydrolysis of collagen and that they were related to absorption and release of silica compounds (Bierich, 1924).

Chemical analyses of the substance (elastin) which presumably represents the elastic tissue have been made in detail on ligamentum nuchae of oxen. Table 1 is taken from Cohnheim's textbook, "Chemie der Eiweisskörper," and table 2 includes the figures obtained by Chittenden and Hart. The comparison of elastin with collagen discloses certain important differences. An interpretation of these differences may aid in the explanation of some of the qualities of the elastica, namely, the acidity, the resistance to acids and the reducing power. These properties are those which were utilized in the development of differential staining methods. Elastic tissue contains only small amounts

of the basic diamino acids, and it is assumed that for this reason acids are less injurious to elastin than to collagen. The principal acid constituents of collagen are aminoacetic acid, glutamic acid, proline and oxypyrrolidin carbonic acid, while those of the elastica are aminoacetic acid, leucine, alanine and phenylalanine. These are less neutralized by basic amino acids, particularly lysine and arginine, than are the acids of collagen. Elastin also differs from collagen in that it has a strong reducing power. This has been attributed by Unna to its relatively high content of reducing amino acids.

Now that the physical properties and some chemical components of elastic tissue have been mentioned, the term "elasticity" may be defined, some of the laws which govern elasticity of solid bodies may be enumerated, and the rules which should be fulfilled to allow for elastic qualities in colloids may be considered. *All this is done for the purpose of approaching the problem as to how well elastic tissue has been designed for the optimal performance of certain functions.*

In the beginning, one must broaden one's views to include not only a perception of elasticity in the physical sense but also elasticity in the common sense. By physical definition, elasticity is the property in virtue of which bodies resume their original form or volume when the force which altered that form or volume ceases to act. By this definition the coefficient of elasticity of bone is much greater than that of elastic tissue. In the common conception of the term "greatness of elasticity" implies the property of great deformation when a force is applied, with almost prompt recovery of the original form or volume after the deforming force has ceased to act. Therefore "great elasticity," as a term of usage in this paper, implies qualities similar to those of a highly extensible rubber band.

Elasticity may be developed in bodies by pressure, traction, flexion or torsion. There is a limit of elasticity of solids beyond which they either break or are incapable of regaining their original form or volume. When the limit of elasticity has not been exceeded, the traction of rods and wires is subject to the following laws:

1. Rods and wires possess perfect elasticity; i. e., they assume their original length as soon as traction ceases.
2. For the same substance and the same diameter, the elongation is proportional to the force of traction and to the length.
3. For rods and wires of the same length and substance, but of different magnitude, the elongation is in inverse ratio to the squares of the diameters.
4. According to calculation and experiment, when bodies are lengthened by traction, their volume increases.

The laws of elasticity of flexure, which apply to bending and return to original form, and the laws of torsion need not be given in detail, but it is reasonable to believe that they may be applied to the elastic fiber.

In addition to the physical laws of elasticity, and inasmuch as the elastic fiber is generally believed to offer resistance to force, the rules of tenacity should be applicable. Tenacity is the resistance which bodies oppose to traction. It is directly proportional to the breaking weight and inversely proportional to the area of a transverse section of the wire. Tenacity diminishes with the duration of traction. A small force continuously applied will often break a wire which would not at once be broken by a larger weight. A cylinder has greater tenacity than a prism. The quantity of matter being the same, a hollow cylinder has greater tenacity than a solid one. In general for all bodies, the tenacity and elasticity are greater in the direction of the fibers than in a transverse direction. For most bodies, tenacity rapidly decreases as the temperature is increased.

There seems to be no valid reason for believing that elastic tissue does not obey these rules and laws. In this regard the wide differences in the texture and structural arrangement of elastic tissue may be emphasized. The application of these laws to the anatomic structure and distribution of the elastica becomes difficult because of the complications which arise when one attempts to analyze the part played by other elements, especially collagen and smooth muscle, with which the elastica is so intimately integrated. Nevertheless, a crude survey of the development, the localization and the variation in form and arrangement of elastic tissue shows how well this tissue has been designed to diffuse the forces of applied stresses, to allow for fluctuation in the shape of organs, to guard against the ill effects of excessive forces and to expedite the return of the deformed structure to its natural state. A complete dissertation on this subject could be given only after most extensive studies. Such studies have not been made.

To supplement the possible application of physical laws as outlined one may turn to an analysis of the factors which, according to Busse, govern the property of high elasticity in elastic colloids. First, there must be groups of atoms which form somewhat flexible fibrous units. Most requisite to this factor are long fibers. Of these, elastic tissue has an abundance, for, as has been seen, the continuous networks exhibit no free ends, and the elastic fibers may be traced for great distances without interruption. This is particularly true in the vascular system. Secondly, the fibrous unit of the elastic colloid must have weak or uniform cohesive (secondary valence) forces around them. From the roentgen ray spectrograms it has been possible to assume that

the elastic fiber has weak secondary valence forces. Thus, in this respect, also, it is designed for high elasticity. Furthermore, on morphologic grounds it may be concluded that the fibers, as a rule, are so related to neighboring elements that they are enabled to stretch and retract with minimal hindrance when such movements are necessary. Thirdly, the highly elastic colloid must be composed of a three dimensional network which is formed by chemical combinations, secondary valence forces or mechanical entanglements. In elastic tissue the first means of interlocking the fibrous units, if present, is unknown. Roentgen ray analysis gives some reason for believing that there are weak secondary valence forces. The third means, which is of great importance, is exemplified by the fundamental structural arrangement of the continuous meshwork of elastic fibrils. Fourthly, colloids of great elasticity must have a means by which free energy is stored during the process of deformation, to be utilized in the performance of external work during the process of recovery to the original form or volume. It is theoretically plausible that the means by which the elastic fiber stores energy on deformation may be through a distortion of molecules, as evidenced by the assumption of doubly refractive qualities on stretching, with consequent accumulation of potential energy which is available for doing work. What part the reducing factors of certain components of the elastic fibril may play is a problem which remains unsolved. There is no reason to believe that this chemical characteristic is requisite for elasticity of the fiber. From the data given it may be inferred, therefore, that the elastic fiber fulfils so far as is allowed by the limitations of the available data those requirements by which bodies achieve high elasticity.

STAINING METHODS AND REACTIONS

It is not advisable to give detailed accounts of the various technics of staining. It seems more appropriate to outline the evolution of staining methods, to evaluate their usefulness, to describe normal and pathologic reactions and to present theoretic explanations for the affinity of elastic tissue for certain "specific" stains. Any assumptions which might arise concerning the functional integrity of the elastica based on arbitrary criteria of staining reactions should be tempered by the fact that optical properties are characteristic before the development of an affinity for specific stains. Furthermore, a survey of the staining reactions of elastica in pathologic states indicates that an abnormally intense or a weak reaction may not necessarily have the great significance that many authors have assumed. Neither are normal reactions exact criteria by which function may be measured.

The physical and chemical differences between elastic tissue and collagenous tissue are the means by which microscopists have been enabled to distinguish the two types of tissue and to perfect differential stains. The physical structure and especially the optical properties were utilized by the earliest histologists in recognition of elastica. In later years the chemical differences became more apparent. These gave origin to two terms, "elastin" and "collagen." The former was used to designate the chief substance of elastic tissue, and the latter, of fibrous connective tissue. It was discovered that elastin was more acid than collagen, that collagen swelled in acid solutions while elastin did not, and that elastin had certain strong reducing characteristics which were not possessed by collagen.

Virchow (1851) found that when tissues were treated with acetic acid the collagenous fibrils became swollen and thereby could be distinguished from elastic fibrils, which were unaffected. The swelling of collagen in acids and the inherent acid character of elastin were the observations on which early staining methods were founded. These methods consisted in the utilization of acids, such as nitric acid (Taenzer) and sulfuric acid (Manhot), as well as of basic dyes, such as fuchsin or safranin.

The reducing power of elastin was used in the further development of staining methods. It was discovered that elastic tissue when treated with potassium permanganate becomes dark brown and thereby is differentiated from collagen. Also, in contrast to collagen, elastin attracts and reduces the highly oxidized metallic acids, such as osmic acid and chromic acid. By this means the acidity of elastin may be increased so as to enhance its affinity for basic dyes. On the principle of the reducing power of elastin, the two most useful stains were developed. These are the orcein stain (Taenzer-Unna) and the resorcin-fuchsin stain (Weigert).

The theory of the Weigert stain as given by Unna (1928) is as follows: The acid elastin has a chemical affinity for basic fuchsin. Added to this is the affinity of the "reducing" elastin for an oxidizing agent, ferric chloride. This reaction is enhanced by treatment with the bivalent phenol, resorcinol, which is a strong reducing agent. The use of resorcinol is not essential, because fuchsin and ferric chloride will stain elastin in a specific manner. Ferric chloride may be replaced in this reaction by other oxidizing agents, such as ammonium persulfate, chromic acid and potassium dichromate. The advantage in using ferric chloride is that the reduction of this chloride by elastin leads to the formation of hydrochloric acid, an acid which causes collagenous fibrils to swell, thereby inhibiting their affinity for the basic dye fuchsin.

Although the Weigert stain is very satisfactory, many authors have given preference to the orcein stain (Taenzer-Unna) because other

differential stains can be used at the same time, because the detail of delicate fibrils is somewhat better and because the Weigert stain is useless for the differentiation of elastin and elacin. Orcein is a pure acid dye that combines with the acid substance elastin in an oxypolar manner. This combination is brought about through the capacity of elastin to reduce orcein. The addition of nitric acid is necessary because of its effect on the collagen. Numerous variations of the orcein and resorcinol-fuchsin stains have been developed. In general, the basic principles are the same. No attempt will be made to describe the numerous modifications, but certainly the Verhoeff stain is very reliable, and many believe that there are advantages in its use.

Elsewhere in this review it has been shown how the normal "mature" staining reactions of elastic tissue gradually developed, phylogenetically in the animal series, ontogenetically in man and culturally in studies *in vitro*. So far as I am aware, no experimental evidence has been brought forward which will explain the increasing intensity of the so-called specific staining reaction. Any information in this regard should shed some light on the means by which elastic tissue attains certain singular capacities. It must be kept in mind that in some instances the colloid in the thyroid gland, mucin, collagen and basement membranes may elect the specific stains. Otherwise, for practical purposes the orcein and resorcinol-fuchsin stains demonstrate the normal elastic tissue in a satisfactory manner.

The alterations which occur in the elastica may be distinguished not only by atypical reactions to the specific stains but also by various structural changes. It may be emphasized here that a change in the physical properties or in the chemical structure of the fiber does not necessarily imply that there is a comparable change in the uniformity or intensity of staining. Neither does a change in the staining reaction signify in all instances a parallel change in the functional ability or in the structural integrity of the fiber. Therein lies the weakness of many dogmatic interpretations of histologic appearances.

Most authors have expressed the belief that the first stage of atrophy is a partial or complete loss of elective staining qualities. The first fibers to be involved are the delicate fibrils. At the same time the larger fibers become separated into segments, often with alternation of stained and unstained parts. Following this they lose their specific staining qualities, diminish in thickness and eventually disappear. Krösing and Passarge contended that this was true because, as they showed, in tuberculous areas where the elastica had largely disappeared treatment of the tissues with potassium hydroxide enabled them to visualize elastic fibers that previously could not be distinguished. Unna (1896) noted that some of the fibers of senile skin were stained weakly

with orcein. These had an affinity for basic aniline dyes. He used the term "elacin" for the changed substance of these fibers to distinguish it from elastin of normal fibers. Also the senile skin contained a material which had the structure of collagen and the staining qualities of elastin. He called this material "kollastin." There also was an element which had the staining qualities of elacin and the structural arrangement of bundles of collagen. He named this "kollacin." The significance of these elements is unknown. In general, modern authors have accepted the descriptive terms "elastin" and "elacin." The various types of fibers may be found in numerous pathologic states, and due consideration will be given them elsewhere.

The elastic fiber may give evidence of the effect of disease processes in many other ways. It is common for networks to become disrupted and to form skeins, tangles and more or less homogeneous masses. The fibrils may swell, either diffusely or in such a way that the varicose thickenings become so spaced as to resemble a rosary. They may fracture, usually transversely, and curl at their ends. Rarely, they are split longitudinally. They tend to retract spirally and flex to form symmetric arcs. They may break up into a series of refractile elastic granules, reminiscent of the embryonic prefibrillar granule stage which has been described by many authors. These granules may occur in strands, gather in clusters or fuse to form homogeneous masses (Schmidt). Vacuoles which contain fat may be found in the fibrils (Jores, 1902). Therefore, let it suffice for the present to say that the elastic fiber may undergo slight or profound morphologic changes with or without loss of the specific staining reactions of its component parts and that the staining reactions may vary with or without changes of a morphologic nature.

PHYSIOLOGY

The methods which have been applied to the investigation of the function of elastic tissue have given results which as a rule will not bear close scrutiny. In the first place, authors have couched their results either in generalities or in terms of resistance, retraction, extensibility or coefficient of elasticity. In the second place, elastic tissue that was entirely devoid of other conflicting tissue elements has not been used in the experiments. As a result, confusing and contradictory findings have burdened the literature.

Direct observation of the isolated elastic fiber has led to general acceptance (Maximow; Sobotta) of the supposition that the fiber is highly extensible and has inherent ability to return promptly to its natural size and shape. Despite the accepted belief that elastic tissue is of great importance because of its elastic qualities, several authors, especially Sternberg and MacLeod, have contended that the chief func-

tion of the elastic network is one of support—a restraining structure designed to prevent overdilatation of tissues.

Much of the dissension has arisen because of opinions which have been founded on the interchangeability of staining reactions of elastic tissue, collagen and basement membranes. This is dependent on a concept of a theoretic substance, elastin. This concept, as has been stated, embodies the belief that fibers may be impregnated with this substance and that they thereby assume elective staining qualities. The hypothetical impregnation and disimpregnation of collagenous and elastic fibers with hypothetical preformed materials, elastin and collagen, have given rise to such terms as "elastin," "elacin," "collastin" and "collacin." The elastic properties of elacin, collacin and collastin are unknown, but it reasonably may be assumed that the staining reaction is not acceptable as an absolute measure of their functional capacity. Basement membranes, which often stain similarly to elastic tissue, are not necessarily endowed by this staining property alone with the same functional capacity as elastic tissue. Nevertheless, these membranes, as well as collagen and reticulum, possess certain elastic qualities. How these compare with the elastic qualities of elastic tissue remains a question that has not been answered.

Tripel contended that the elasticity coefficient when distending forces were used was greater for collagen than for elastic tissue. When shearing forces were applied, the elasticity coefficient for elastic tissue was greater than that for collagen. By no means do these findings aid one in a solution of problems involving the question of great extensibility and retractile power, for, as is well known, bone has a higher elasticity coefficient than either collagen or elastic tissue.

Certain studies have been made on the elasticity of organs. An organ or tissue containing elastic fibers does not attribute its elasticity to elastic fibers alone. If an organ or tissue is so constructed as to allow for great deformation, a fairly prompt recovery of that organ to its natural size implies that most of its mobile component parts are capable of withstanding great deformation, subsequently returning to normal form. The ability of the organ to resume its natural size depends largely on its structural units. Abnormality of any one of the important units may alter the return of the organ to its natural size and shape by interfering with or enhancing the function of the active elements. The most important active elements are of muscular nature. Cardiac and voluntary striated muscle may be largely disregarded. Smooth, or involuntary, muscle, which is so widely distributed, plays a major role by virtue of its extensibility and its contractile power. Intimately associated with this tissue in so many situations is elastic tissue. Indeed, this association is often so pronounced that the term "myoelastic tissue" has been utilized to designate the combination.

Furthermore, in certain situations the elastica occupies positions where one might well expect smooth muscle. The integration must signify something more than simple support of smooth muscle by the elastica. In the first place, the continuous elastic network is so placed as to aid in the dissemination of stresses directed at isolated points. Secondly, the network is ably designed for coordination of rhythmic movements of separate units. Thirdly, it aids in the conservation of energy through partial maintenance of tone during relaxation of the muscular elements. Fourthly, because of its great tenacity and the continuous character of the membranes and networks, it serves as a bulwark against the possible injury of excessive forces. Fifthly, because of the relatively great extensibility and inherent power of prompt retractility of the elastic fiber, it aids materially in the return of a tissue or organ to the natural form after a deforming force has been removed.

The roles of the remaining primary tissue elements, such as collagen, reticulum, mucin, cartilage, bone and cell structures, are indispensable. All must play some part in the return of distorted organs or tissues to natural forms, but because of basic physical properties or because of anatomic arrangement they become of secondary value in the general scheme. Among these, collagen is of greatest importance because of its almost constant neighborly association with elastic tissue. Thus the physiologic or the pathologic state of one is almost always reflected by functional or structural changes in the other. With this in mind and with the knowledge that pathologic processes usually affect both elements simultaneously, the interpretation of changes in elasticity of tissues must be guarded.

In relation to arteries it has been said that the amount of elastica roughly parallels physiologically the pressure within the artery (Maximow and Bloom). Sternberg stated that the number of fibers in a given tissue is not an absolute index of the degree of elasticity of that tissue. As an example, he cited arteries of the muscular and of the elastic type. Although there is little difference in the elasticity of these two types of vessels, there is great difference in the amount of elastica.

The most illuminating and careful researches dealing with the physiologic importance of elastic tissue have been conducted by the school of English physiologists. Their studies have been concerned chiefly with the function of the elastica of blood vessels. I have drawn liberally from the excellent presentation of this work as given by Bramwell.

It has been learned that arteries may be divided on the basis of their constituent elements into vessels of the elastic type, vessels of the muscular type and vessels of a transitional structure. Typical elastic arteries are the aorta, the subclavian artery and the carotid artery. The

muscular arteries may be exemplified by such vessels as the radial and the lingual. The axillary and the common iliac arteries may serve as examples of the transitional forms. It has been shown that under normal conditions the smaller arteries, such as the radial, are less elastic than the larger vessels, especially those of the "elastic" type (Bazett and Dreyer; Fulton and McSwiney). It has been learned that there are certain diseases which affect the vascular system in such a manner as to reduce the elasticity of vessels. The vessels which are affected in these conditions and the degree of their functional impairment are such as to exert an important influence on the circulation of the blood. For this reason this subject will be given further consideration in the following paragraphs.

The efficiency of arteries as judged from one point of view must depend principally on their elastic qualities. During the initial phase of ventricular systole the cardiac output is accommodated largely by distention of the aorta. The rapid stretching of the aorta and other arteries allows for a storage of potential energy in the vascular wall. This energy is utilized during the period of retraction in promoting the forward movement of the blood column. The greater the elasticity of arteries the lower will be the pressure required to produce a given increase in volume for the accommodation of the cardiac output. Since the energy expended by the ventricle in systole is proportional to the pressure developed, the effort of the ventricle will vary inversely with the extensibility of the arterial walls. Similarly, during the phase of arterial retraction the more elastic the arteries the smaller is the fall of pressure for a given decrease in their volume. In other words, the more elastic the arterial walls the lower is the pulse pressure and the more uniform the blood flow through the capillaries. The conclusion may be reached that the greater the alteration in volume of arteries in response to a given alteration in pressure the more efficient is the arterial mechanism.

Since the velocity with which the pulse wave is transmitted along arteries depends principally on the elasticity of the arterial walls, direct observations on the velocity of the pulse wave have been used as a basis for the calculation of the mean extensibility of vessels in absolute units (Bramwell and Hill). Bramwell, Hill and McSwiney showed that in normal healthy persons the velocity of the pulse wave increased with age. They found that the elasticity of arteries was halved between 10 and 60 years of age. Comparable results were obtained from a study of arteries removed from subjects post mortem. This variation in arterial elasticity may be attributed principally to structural changes in the arterial wall even though, as has been stated previously, these changes are not always demonstrable by histologic study.

In a study of isolated human arteries it was found that at varying internal pressures, as the diastolic pressure was increased a disproportionately large superimposed pressure was necessary to produce the same percental increase in the volume of the vessel. The tension with which the artery was stretched longitudinally did not affect the circular elasticity to any appreciable degree. The results were explained by the hypothesis that the arterial wall consists of a series of different elastic systems. At very low pressures only the most distensible of these are stretched. As the pressure rises, less and less distensible systems are called into play. At low pressures the artery exhibits a high degree of elasticity. At higher pressures the pulse velocities become a linear function of pressure since the vessel becomes relatively nonelastic.

Wilens made a careful study of the postmortem elasticity of the aorta. He found that restraint of the intima often disturbed measurements of elasticity, and he eliminated this source of error by stripping the intima from the media. The results of his measurements show that postmortem elasticity is almost exclusively a function of age and is relatively constant in any given age group. The mobile parts of the aorta retain more elasticity than the fixed parts.

There is little relationship between intimal lesions and loss of elasticity of the regional media. The elasticity is approximately equal in longitudinal and transverse directions of all segments of the aortic wall.

It is interesting that in certain wasting diseases the elastic element of the arterial wall appears to lose much of its efficiency (Bramwell, Downing and Hill). This finding is comparable to similar results obtained from a study of the elasticity of the skin by Schade (1912). Furthermore, hypoextensible and hyperextensible arteries have been found in apparently normal subjects. Such individual variations also occur in the elasticity of the skin and possibly in that of the lungs of normal people.

The elasticity of the lungs, which are rich in elastic tissue, is of great importance in respiratory mechanics. This elasticity does not change materially for several days after death (Bönniger, 1908). The great increase in the volume of lungs during inspiration takes place mainly through distention of alveolar ducts and to a lesser degree through distention of the bronchi and bronchioles rather than through that of alveoli (Maximow and Bloom). These parts of the lungs which have greatest fluctuation in size during normal respiration have the richest supply of elastic tissue. In a previous part of this article it has been stated that the full development of the pulmonary elastica is not attained until the lungs have been used for respiration. According to Cloetta, the expiratory movement is a purely elastic phenomenon, and the collapse of the lung in pneumothorax is due more to retraction of the elastica, which is under constant tension, than to closure of the

small bronchi. On the basis of experimental studies Cloetta concluded that for expansion during inspiration the lung possesses ideal elasticity. Bönninger found that the lungs of children were more elastic, in the commonly accepted sense, than those of adults. This roughly parallels the conception of the elasticity of the skin and arteries.

The skin because of its availability has received more study, and an effort has been made to relate the changes in the elasticity of the skin to various local and systemic pathologic processes (Bönninger, 1904-1905). An excellent discussion of methods of measuring elasticity is given by Reuterwall. The resistance of the skin to palpation has served as a crude index of elasticity, but Schade devised an instrument by which the relaxation time of the skin could be measured. This mechanical device was called an elastometer, and the application was known as elastometry. By this method of study Schade found that a disturbance in elasticity was in general an early symptom of organic disease. He obtained low values in diabetic acidosis, myxedema, pernicious anemia and septic fevers. In septic fevers the values were 12 to 26 per cent below normal. It may be interesting to speculate on the effect of changes in elasticity in other organs, if one assumes that they may parallel cutaneous changes. Such a change has been suggested as affording a partial explanation of the soft splenic tumor which often accompanies infection in the adult (Lubarsch). Schade (1912) found that elasticity of the skin diminished with increasing age and was least in old persons. In edema of the skin elasticity was reduced even before edema was palpable. The ratio between the degree of edema and the loss of elasticity was not constant, but the average reductions varied from 20 to 50 per cent. In inflammatory edema the diminution in elasticity was of similar degree, and Schade (1912) believed that comparable losses were probably present in lungs affected by pneumonia.

The effect of fatigue on the elasticity of connective tissues has been studied. Schade (1921) found that a sleepless night reduced the elasticity of the skin about 15 per cent. Katzenstein measured the amount of fluid which was necessary to fill knee joint capsules of rabbits before and after exercise of the joint. He demonstrated that prolonged active and passive movements of the joint were followed by a substantial increase in the capacity of the joint capsule. It was assumed that this was the result of relaxation in the fibers of the joint capsule.

(To Be Concluded)

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, Etc.—T. Wingate Todd, professor of anatomy in Western Reserve University and director of the Brush Foundation, Cleveland, died Dec. 28, 1938, at the age of 53.

A. F. Bernard Shaw has been appointed to the joint post of professor of pathology in the University of Durham and pathologist to the Royal Victoria Infirmary, Durham, England, in succession to Stuart McDonald.

Karl Sudhoff, the medical historian, died Oct. 8, 1938, in his eighty-fifth year.

According to the *Lancet*, Phillipp Schneider is the new director of the Institute of Forensic Medicine in Vienna.

Ernest E. Tyzzer, professor of comparative pathology in Harvard Medical School, has been appointed professor of tropical medicine also, succeeding Richard P. Strong, retired.

Henry Pinkerton, assistant professor of pathology at Harvard Medical School, has been appointed professor of pathology in St. Louis University, St. Louis.

In the Pasteur Institute, Paris, Harry Plotz has been placed in charge of virus research.

G. B. Magrath, professor emeritus of legal medicine in Harvard Medical School, died Dec. 10, 1938, at the age of 68 years.

Harvard Symposium on Viruses.—The Harvard School of Public Health offers a course of lectures, clinics and demonstrations on the virus and rickettsial diseases, with special emphasis on their significance for public health; June 12 to 17, 1939. Lectures on the etiology, epidemiology and methods of control of these diseases, by members of the faculties and by former students of the Harvard School of Public Health and of the Harvard Medical School, will occupy five mornings. Special clinics and demonstrations will be given each afternoon. On the last morning, a panel discussion will be held on the three main topics presented in the symposium. The fee for the course will be \$25. Enrolment should be arranged before June 1, as facilities for many of the clinics and demonstrations are limited. The lectures will be published later in a single volume, which will be sent to each registrant for the course. For further information, write to the Secretary of the Harvard School of Public Health, 55 Shattuck Street, Boston, Mass.

Society News.—The eighteenth annual meeting of the American Society of Clinical Pathologists will be held in St. Louis, May 12, 13 and 14, 1939. The Hotel DeSoto will be the official headquarters.

The American College of Physicians will hold its twenty-third annual session in New Orleans, March 27 to 31, 1939.

Celebrations.—The sixtieth birthday of Howard T. Karsner and his twenty-fifth year as professor of pathology in Western Reserve University were celebrated Jan. 6, 1939, when a portrait of Dr. Karsner was presented to him.

Abstracts from Current Literature

TO SAVE SPACE THE ORIGINAL TITLES OF ABSTRACTED ARTICLES SOMETIMES
ARE SHORTENED

Experimental Pathology and Pathologic Physiology

DERMATOSES DUE TO VITAMIN A DEFICIENCY. J. B. YOUMANS and M. B. CORLETTE, *Am. J. M. Sc.* **195**:644, 1938.

A dermatosis due to deficiency of vitamin A is described, with the histologic studies. Some of the patients presented the dry horny papular lesion described by Frazier and Hu; others presented an acne-like lesion that differed in some respects from those previously described. The histologic picture and the response to treatment with vitamin A were similar in the two types of eruption.

The relation of these changes in the skin to the other manifestations of avitaminosis A is of considerable importance. Xerophthalmia and hemeralopia were present in most of the cases reported by Loewenthal and in many of those reported by Frazier and Hu. Cornification of the epithelium of the conjunctiva was observed by the latter in patients with less severe lesions of the eye. Nicholls' patients had night blindness. In none of the patients whom Youmans and Corlette studied were there changes in the epithelium of the eye, and only occasionally did one give a history of mild night blindness, and then the history was questionable. Studies with a visual photometer demonstrated in some of the subjects occasional mild night blindness. Blackfan and Wolbach and others showed that the manifestations of a lack of vitamin A are evident in the epithelium of many tissues and organs and that these changes vary in respect to the order of their appearance and their severity. Although the changes in the eye and in the vision have been thought to be the earliest reliable clinical manifestations of avitaminosis A, the observations of Youmans and Corlette, as well as those of Frazier and Hu, suggest that in some instances the cutaneous lesions may be among the first clinical evidences of the deficiency, appearing before demonstrable changes in the epithelium of the eye and before more than a mild night blindness, detectable only by a photometer, is present. If so, these changes may constitute one of the earliest signs of vitamin A deficiency, fortunately easily recognizable.

FROM AUTHORS' SUMMARY.

RENAL INSUFFICIENCY FROM BLOOD TRANSFUSION. E. L. DEGOWIN, E. D. WARNER and W. L. RANDALL, *Arch. Int. Med.* **61**:609, 1938.

The transfusion of canine hemoglobin into dogs when the urine is acid results in death from renal insufficiency. This does not occur when the urine is alkaline at the time of the transfusion. The anatomic picture of obstruction of the renal tubules by hemoglobin pigment sufficient to be the chief cause of the renal insufficiency is observed in most dogs under the experimental conditions outlined. A nephrotoxic process often operates and may cause renal insufficiency independently. The deposition of hemoglobin pigment as hemosiderin in the renal tubules and in the reticuloendothelial system apparently does not contribute to the development of renal insufficiency. An anatomic study of the kidneys of 9 human beings who died of renal insufficiency after hemolysis revealed the two independent mechanisms seen in dogs, the obstruction with pigment and the necrosis. In occasional human beings the precipitation of hemoglobin pigment in the tubules is extensive and may be a cause of renal insufficiency. This complication could probably be prevented by alkalinizing the urine prior to the transfusion. In the majority of human beings showing renal insufficiency after hemolysis the condition is probably caused by some nephrotoxic substance which causes degeneration of tubular epithelium and interstitial edema.

FROM AUTHORS' SUMMARY.

CHANGES IN THE BRAIN IN PLEXECTOMIZED DOGS, WITH COMMENTS ON THE CEREBROSPINAL FLUID. G. B. HASSIN, E. OLDBERG and M. TINSLEY, *Arch. Neurol. & Psychiat.* **38**:1225, 1937.

The authors tried to determine the effect of removal of the choroid plexus on the size of the cerebral ventricles and the changes that take place in the brain in such experiments. In some animals the sylvian aqueduct was blocked without removal of the choroid plexus; in others the choroid plexus was either removed from one lateral ventricle or left in the latter after closure of the ipsilateral foramen of Monro with a piece of fascia and muscle; in another group both choroid plexuses were removed after blocking both the foramina of Monro. Plexectomized brains invariably exhibited connective tissue scar formation, degenerative, reactive and inflammatory phenomena, ependymitis and subependymitis, which may affect the size of the ventricles. It was not possible to remove the choroid plexuses in toto, for small fragments of them could be demonstrated under the microscope. The results of plexectomy were by no means uniform, as in some cases a plexectomized ventricle with the foramen of Monro blocked appeared to be of the same size as the opposite ventricle or smaller or even larger. Like pathologic observations on hydrocephalus in man, those following experimental plexectomy force one to the conclusion that the cerebrospinal fluid accumulates in the ventricles but is derived not from the choroid plexus but from the tissue fluids of the brain, which are drained by the ventricles and subarachnoid space. It is not an organ of secretion but of excretion, eliminating from the spinal fluid substances which are harmful to the nervous system and which render the fluid more absorbable.

GEORGE B. HASSIN.

BLEEDING TENDENCY AND PROTHROMBIN DEFICIENCY. H. P. SMITH and others, *J. Exper. Med.* **67**:911, 1938.

In dogs with biliary fistula the plasma prothrombin falls eventually to low levels, and bleeding commonly occurs. An important causative factor in this fall is the faulty absorption of vitamin K from the intestine in these animals. Feeding bile permits absorption of the traces of this vitamin normally present in mixed diets, and, as a result, a slow rise in prothrombin is observed. If a standard diet is supplemented with large amounts of vitamin K concentrate, the rise is rapid, provided bile or bile salt is supplied to aid in the absorption. Variations in the rate of depletion of prothrombin in dogs which have biliary fistula and which are kept on a constant diet indicate the existence of additional factors which require further study. The experience of Smith and his co-workers indicates that vitamin A and vitamin D supplements do not correct the prothrombin deficiency in animals with biliary fistula.

FROM AUTHORS' SUMMARY.

PERIPHERAL BLOOD PHENOMENA AND DIFFERENTIAL RESPONSE OF BONE MARROW AND LYMPH NODES TO HYPERPYREXIA. C. A. DOAN, *Radiology* **30**:382, 1938.

The hemopoietic response to "fever" is rather constant, and the majority of the cells making up the postfebrile leukocytosis are polymorphonuclear neutrophils, newly delivered by the bone marrow, as shown by their youth. This part of the reaction may be nonspecific and is by no means necessarily the most important from the standpoint of the fundamental defenses of the body. There is destruction of lymphocytes during hyperpyrexia, as attested by the studies of lymph nodes cited and by the return of very young cells to the circulation after prolonged lymphopenia. There is probably in the human patient some destruction or redistribution of monocytes, as is shown by the delayed monocytosis, made up primarily of younger forms. The hemograms made after inoculations of malarial parasites and of typhoid vaccine differ from those observed during fever induced by physical methods in the marked leukopenia during the chill, in the temporary disappearance of the monocytes from the circulation following typhoid and in the marked stimulation of the monocytes in malaria and their moderate stimulation following

inoculation of typhoid vaccine. The shift to the left in the neutrophilic granulocytes in malaria is outstanding, and the appearance of clasmotocytes in the peripheral blood has been observed with no other type of experimental fever. It has been suggested by Breutsch that the profound stimulation of phagocytic clasmotocytes observed in malaria as the result of the destruction of red blood cells by plasmodia provides an important cellular defense weapon in the treatment of syphilis of the central nervous system, which is not available when other fever-producing methods are employed. Cunningham has emphasized the importance of clasmotocytes in the control of experimental syphilis in rabbits. While it is true that biopsies of the sternal marrow in the human patient cited, and the bone marrow of rabbits studied post mortem following hyperthermic fever therapy did not show an increase in clasmotocytes, there was a tremendous increase in these phagocytic cells elsewhere in the tissues, more especially in the lymph nodes, spleen and liver. To that extent, at least, hyperthermia, produced by physical means, not only provides the thermal factor of importance for the inactivation of *Spirochaeta pallida* and the gonococcus but, as now demonstrated, exerts a profound effect on the cellular equilibriums of the body—in the directions which, it is believed by the author, are the most effective in mobilizing the defense forces of the body against these organisms. In short, hyperpyrexia acts as a two-edged sword cutting both ways in its role as "assistant extraordinary" to the humoral defense mechanisms of the body.

FROM AUTHOR'S SUMMARY.

THE EARLY LESIONS OF EXPERIMENTAL ENDOCARDITIS. W. DIETRICH, Virchows Arch. f. path. Anat. **299**:285, 1937.

On the theory that sensitization causes an increase in the reactivity and resorptivity of all the tissues, rabbits were sensitized by repeated injections of horse serum, caseosan (a product which is essentially a 5 per cent solution of casein), colon bacillus vaccine and histamine. After sensitization suspensions of living colon bacilli or of staphylococci were injected intravenously; these injections were repeated in the course of four to five days. At intervals of seven to seventeen days later the animals were killed and the cardiac valves examined microscopically. The earliest changes noted were swelling of the endothelium and varying degrees of edema of the subendothelial tissue, together with moderate cellular proliferation of this layer. Bacteria in small numbers were found in the endothelium and in phagocytic histiocytes of the subendothelial tissue. A thin layer of fibrin was often deposited on the surface of the endothelium; bacteria were deposited in this fibrin layer and multiplied in it. Horse serum and caseosan evoked a more marked reaction than the other sensitizing substances used. When living colon bacilli were used for the provocative injection, the slight, early changes described resulted. Living staphylococci led to acute ulcerative endocarditis.

O. T. SCHULTZ.

NARCOSIS AND HYPERERGIC INFLAMMATION. W. EICKHOFF, Virchows Arch. f. path. Anat. **299**:300, 1937.

To determine whether narcosis, which prevents the onset of anaphylactic shock in sensitized animals, also affects the hyperergic vascular inflammatory reaction, rabbits and guinea pigs were sensitized with swine serum. Under deep and prolonged narcosis the animals received one or more large intravenous or intracardiac provocative doses of the antigen. The prevention of immediate symptoms of shock was found to depend on the depth and duration of the narcosis. Rabbits anesthetized by the inhalation of ether escaped immediate shock but lived at the most ten hours. The lungs of such animals were deeply congested and revealed early pneumonic changes, ascribed to the cooling and irritant action of the anesthetic. Animals narcotized by the intravenous injection of ethyl carbamate (urethane) did not show anaphylactic shock; they recovered from the narcosis and appeared well. Such animals were killed at intervals of eight, fourteen and

twenty-one days after injection of the provocative dose of the antigen. Animals that lived fourteen days or longer revealed the perivascular histiocytic and leukocytic inflammatory reaction, similar to that of periarteritis nodosa, considered to be the characteristic hyperergic effect on the vessels. Ethyl carbamate narcosis delayed but did not completely prevent hyperergic inflammatory reaction. Vascular reaction was most marked in the heart, liver and kidneys. Changes in the hydrogen ion concentration of the blood and in the vascular regulatory mechanism of the autonomic nervous system are believed to be factors in the effects of narcosis.

O. T. SCHULTZ.

Pathologic Anatomy

ABNORMAL DISTRIBUTION OF THE SUPERFICIAL MUSCLE BUNDLES IN THE HUMAN HEART. J. S. ROBB and R. C. ROBB, *Am. Heart J.* **15**:597, 1938.

Fifty human hearts have been dissected to demonstrate the ventricular muscle bands. The superficial and deep sinospiral and bulbospiral muscles were present in all hearts. The authors know of no report in which these muscles were stated to be absent. The surface pattern of these muscles is variable, especially at the lower part of the anterior surface of the right ventricle, in the right ventricle near the conus, along the trabeculated area and at the anterior horn of the left ventricle. The angle at which the superficial sinospiral muscle fibers pass from the anterior horn to the right base varies considerably. In the small heart the fibers have an oblique course, tending to approach a vertical course from apex to base. In the hypertrophied heart these fibers have an almost horizontal course. The masses vary considerably. The right portion of the deep sinospiral muscle is differentially hypertrophied in mitral disease or in any other disease characterized by increased resistance to the flow of the pulmonary blood. The deep bulbospiral muscle is similarly hypertrophied in hypertension and aortic stenosis. If the work of the heart is much increased, the left portion of the deep sinospiral muscle may also hypertrophy.

The surface portions of the two superficial muscles do not have a measurable variation in thickness. When intraventricular pressure is increased, the papillary portions of these superficial muscles hypertrophy. Conversely, in a heart in which the mitral valve has a "buttonhole" opening and calcified leaves the papillary portions of these muscles atrophy. If the surface muscles are variable in distribution, or if they are deficient, apparent discrepancies may occur when one is localizing points of initial negativity, the origin of premature beats and other phenomena. For surface localization of electrical phenomena accurate sketches of the surface distribution of the cleaned muscle should be provided.

FROM AUTHORS' SUMMARY.

THE BONE AND CARTILAGE LESIONS OF PROTRACTED MODERATE SCURVY. A. W. HAM and H. C. ELLIOTT, *Am. J. Path.* **14**:323, 1938.

Protracted moderate scurvy was produced in young guinea pigs by feeding them diets containing less than adequate amounts of vitamin C. It was shown that longitudinal growth of bone continued under this regimen only because most of the limited amount of new tissue which formed was in the peripheral part of the epiphysial plate and in the ring of the diaphysis adjacent to the periphery of the plate. This resulted in weakness of the shaft in this location and nonsupport of the epiphysial plate. The chief manifestation of scurvy in the epiphysis was found to be a diminution in the amount of the bone supporting the articular cartilage. It was pointed out that in the adult whose growth is over the effects of scurvy would become apparent in sites where continual replacement of tissue occurs to compensate for wear and tear. It was suggested that certain features of osteoarthritis (the poorly maintained articular cartilages, the generalized diminution of the amount of bone in the skeleton and the osteophytes) would be

not unlikely effects of a long-continued moderate deficiency of vitamin C in the adult. Lastly the theory which postulates that vitamin C controls the jelling of intercellular substances was discussed in the light of others' findings and those of the authors, and no support was found for this theory.

FROM AUTHORS' SUMMARY.

NATURE OF THE "SILVER CELLS" IN MULTIPLE SCLEROSIS AND OTHER DISEASES.

N. BLACKMAN and T. J. PUTNAM, *Arch. Neurol. & Psychiat.* **39**:54, 1938.

"Silver cells" were described by Steiner, in observations on multiple sclerosis, as small round bodies the size of a lymphocyte. They invariably contained argentophilic small granules of precipitated silver, which Steiner assumed to be phagocytosed spirochetes. Blackman and Putnam found these cells in lesions of the blood vessels of the brain (arteriosclerosis, trauma and hemorrhage) in which the possibility of local phagocytosis of micro-organisms could be excluded. By using additional staining methods on adjacent frozen sections from the same block the authors deduced that the silver cells are of glial origin, that the particles are not necessarily spirochetes and that they are probably of hematogenous origin (calcium, iron).

GEORGE B. HASSIN.

TISSUE SPACES OF THE KIDNEY. F. FUCHS and H. POPPER, *Virchows Arch. f. path. Anat.* **299**:203, 1937.

The demonstration of tissue spaces between the capillaries and the parenchyma of certain organs has introduced the concept of serous inflammation, in which serum escapes into the pericapillary space and may lead to disruption of the capillary, on the one hand, and of the parenchyma, on the other. Such a process in the liver has been termed serous hepatitis and has been described in an article previously abstracted. The present authors concerned themselves with the demonstration of tissue spaces in the normal kidney. The rabbit's kidney and the human kidney, obtained at necropsy, were used. India ink diluted with physiologic solution of sodium chloride (1:4) was injected into the pelvis under moderate pressure by means of a cannula in the ureter. The injected fluid entered the perivenous spaces of the pelvis and passed distally through the parenchyma of the organ. This procedure was sometimes combined with injection of colored gelatin into the arteries. A space filled with the ink surrounds the capillary and separates the latter from the tubule.

O. T. SCHULTZ.

INTERRELATIONSHIP BETWEEN THE LIVER AND THE BRAIN. V. NICOLAJEV, *Virchows Arch. f. path. Anat.* **299**:309, 1937.

The association of changes in the brain and liver in Wilson's disease led to a histologic study of alterations in the brain and liver in various diseases of the latter organ in man and in animals subjected to a variety of hepatic poisons. The results of this study were recently published in Lettish. The present article is an eight page summary of the previously published observations. Degeneration and disintegration of the hepatic parenchyma lead to the formation of "hepatogenic toxins" that have a deleterious effect on the brain, resulting in functional insufficiency of the glia and in degenerative changes, or a status spongiosus, of the glia. The degenerative changes are the result of alterations in the circulation. Such an effect on the brain results from a variety of pathologic processes in the liver. The changes in the central nervous system in Wilson's disease are characteristic, since they develop on the basis of a congenital anomaly of metabolism. Icterus is not a pathogenic factor in the cerebral changes observed.

O. T. SCHULTZ.

FIBRINOID DEGENERATION OF CONNECTIVE TISSUE FOLLOWING A SINGLE INJECTION OF PROTEIN. U. GRAFF, *Virchows Arch. f. path. Anat.* **299**:339, 1937.

Graff criticizes the tendency so evident in the current literature to term hyperergic the inflammatory reaction observed in a constantly increasing variety and number of human diseases on the basis of similarity of alterations produced in the specifically sensitized animal. He attempts to show experimentally that similar changes and especially the fibrinoid degeneration of connective tissue that many hold to be characteristic of hyperergic inflammation can be produced by a single injection of foreign protein and therefore are not evidence of hyperergic reaction. Rabbits and cats were used, but chiefly rabbits, in the relatively small series of experiments reported. Admitting that a change in the reactivity of the vessels is necessary, the ear of the rabbit was warmed in water at 40 C. before injection of the protein, or the latter was preceded by an injection of allylforminate, which renders the vessels more permeable. The animals were killed at intervals of from two hours to fourteen days after the injection of protein, and the tissues were examined histologically. The protein chiefly used was swine serum. Graff describes fibrinoid degeneration and cellular infiltration that he claims are similar to those seen in hyperergic inflammation. He concludes that his results establish the correctness of his thesis that not every so-called hyperergic inflammation is the result of sensitization. Graff's communication is followed by a note by Roessle (p. 359), who had the opportunity of examining Graff's preparations. Roessle severely criticizes Graff's observations and interpretations. He denies that there is identity or even great similarity between the changes produced by Graff and those which competent pathologists consider characteristic of experimental hyperergic inflammation. What Graff terms fibrinoid degeneration is not such. Unusually large amounts of swine serum were injected. This may have had a primary toxic action, or the slow degradation of the serum may actually have sensitized the animals. In Graff's experiments from three to seven days were required for the development of the changes described. This, according to Roessle, is in striking contrast to the rapidity with which allergic hyperergic inflammation develops in the experimental animal.

O. T. SCHULTZ.

PARTICIPATION OF THE LYMPHATICS IN PATHOLOGIC ALTERATIONS OF THE SPLEEN.

E. JAEGER, *Virchows Arch. f. path. Anat.* **299**:552, 1937.

In conditions leading to stasis in the portal system the quantity of lymph drained from the spleen is increased over the normal. The hilar lymph nodes of the spleen enlarge and take on a reddish brown color, which has led to the belief that they become transformed into hemolymph nodes and take over part of the function of the spleen. Although it is difficult or impossible to demonstrate deep lymphatic channels in the spleen by injection methods, dilated endothelial-lined spaces are evident in the periadventitial tissue of the arteries in conditions of stasis. By means of serial section reconstructions Jaeger was able to show that these spaces are part of a network of lymphatic vessels which begin in the malpighian bodies and run in the periarterial tissues to the hilus of the spleen. In venous stasis of the spleen erythrocytes are forced into the lymphatic channels. In the malpighian bodies the deposition of iron pigment leads to the formation of the Gandy-Gamna iron and calcium incrustation bodies. Deposition of the pigment in the hilar nodes transforms these into pseudohemolymph nodes. Periarterial fibrosis of the spleen results from inflammatory reaction on the part of the lymph vessels; it is a chronic perilymphangitis.

O. T. SCHULTZ.

HYPERTONIC APOPLECTIC CEREBRAL HEMORRHAGE. K. WOLFF, *Virchows Arch. f. path. Anat.* **299**:573, 1937.

Following apoplectic cerebral hemorrhage in persons with a prolonged antecedent history of hypertension, a variety of alterations are observed in the arteries

in and about the hemorrhage. These include: angioneclerosis, which accompanies and does not antedate the hemorrhage; a process which the author terms plasmatic destruction of the vessel wall, which precedes the hemorrhage and is associated with older changes in the brain tissue; arteriosclerotic changes that are likewise older than the terminal hemorrhage, and aneurysm subsequent to plasmatic destruction of the wall. Rupture of the diseased vessels leads to massive hemorrhage.

In persons without a prolonged antecedent history of hypertension the hemorrhage originates from many smaller, apparently normal vessels, which leads the author to conclude that functional circulatory disturbances of unknown nature initiate the hemorrhage. The latter is accompanied by angioneclerosis of surrounding vessels. These then give way, and the compact massive hemorrhage results. No other alterations have been observed in the vessels. O. T. SCHULTZ.

CYTOPLASMIC INCLUSION BODIES OF THE HUMAN LIVER. A. TERBRÜGGEN, *Virchows Arch. f. path. Anat.* **299**:775, 1937.

Terbrüggen describes cytoplasmic inclusion bodies that he saw in 36 livers in 1930-1931. They were small and stained readily, and each was situated in a small vacuole. Their nature is not known. Their similarity to degeneration products, extruded nucleoli, virus inclusion bodies and protozoa is discussed. The inclusions are apparently identical with those described by Pappenheimer and Hawthorne (*Am. J. Path.* **12**:625, 1936). O. T. SCHULTZ.

VIRCHOW'S LECTURES ON PATHOLOGY AT WÜRZBURG. R. RÖSSLE, *Virchows Arch. f. path. Anat.* **300**:4, 1937.

The three-hundredth volume of *Virchows Archiv für pathologische Anatomie und Physiologie und für klinische Medizin*, to give it the full title that Virchow selected to indicate the full scope of the new journal, has a brief foreword by Rössle, Virchow's successor as professor of pathology at Berlin and as editor of the journal. Begun in 1847 by Virchow and published uninterruptedly ever since, *Virchows Archiv* is the oldest journal devoted to medical science. Rössle points out that the only scientific journals older that are still being published are the French *Annals of Physics* (1789), the oldest scientific periodical in the world, the German *Annals of Physics* (1799) and Liebig's *Annals of Chemistry* (1834). The entire double number of volume 300, comprising 516 pages, is devoted to 27 articles based on work done in Rössle's institute in Berlin, the post which Virchow had made the outstanding medical position in the world. In addition to numerous halftone illustrations, the volume contains several reproductions of colored drawings reproduced directly on the printed page, a process developed more highly in German publications than in any others.

After his introductory foreword, Rössle proceeds with a contribution of historical interest. It relates to Virchow's period at Würzburg, from 1849 to 1856, when he, a young man of 28 years at the beginning of this period, began attracting students to Würzburg from all parts of Germany. The article is based on recently discovered manuscript lecture notes of students of Virchow at Würzburg and contains numerous excerpts from these notes. Among the students whose notes have come to light and are quoted are Goll and Wilhelm His the elder. The lectures dealt chiefly with general pathologic anatomy, but there are notes of courses in special pathology, especially that of bone. Virchow had already begun to lecture to his students on cellular pathology, a subject which was later elaborated into his famous lectures on cellular pathology at Berlin. Complete publication of the student material that Rössle discusses is promised.

O. T. SCHULTZ.

ORIGIN AND FATE OF LYMPHATICS IN PARABIOTIC ANIMALS. R. RÖSSLE, Virchows Arch f. path. Anat. **300**:31, 1937.

In material derived from successful experiments in parabiosis on rats and mice, Rössle describes the formation of lymph vessels and adds an important contribution on the still unsettled matter of the origin of such vessels. At the line of junction of the two animals the formation of new lymphatics by budding from the original channels can be observed, but this is a process of minor importance. What more particularly concerns Rössle is the development of new lymphatics independently of preexisting vessels. Near the line of junction of the parabions, solid cords and masses of small cells with deeply stained nuclei make their appearance between the bundles of fibrous tissue. Whether these cells come from the nuclei of connective tissue or from wandering cells, Rössle is unable to decide. A lumen develops, and the cells become larger and cuboid, resulting in structures almost glandular in character. These new-formed lymphatics do not come from preexisting ones, and no connection with the lymphatic system of either animal of the united pair ever develops. In animals that live long enough coagulation, thrombosis and organization of the contents of the lymphatics occur, and the vessels become obliterated. This closed lymphatic system usually develops in the cutis and subcutaneous tissue of the larger and stronger parabion near the line of junction. Rössle terms this system a *Saugapparat*, or suction apparatus. It withdraws materials from the other parabion, materials which are foreign to the other partner, which stimulate the formation of the lymphatics and lead to the development of immunity, after which the new-formed closed lymphatics disappear. What Rössle finds more difficult to explain is the development of a closed lymphatic system in each parabion. He suggests that this may be due to alternating periods of dominance of one over the other.

O. T. SCHULTZ.

Microbiology and Parasitology

THE SUBMAXILLARY GLAND VIRUS OF THE GUINEA PIG. F. S. MARKHAM, Am. J. Path. **14**:311, 1938.

Spontaneous infection with virus from the submaxillary glands of guinea pigs is described. The incidence in various local stocks of guinea pigs was found to be from 7 to 74 per cent. Inclusions were found in the renal epithelial cells of 8 per cent of the adult animals examined. Histologic evidence is presented which suggests that the inclusion bodies associated with the virus of the salivary gland of the guinea pig are composed of elementary bodies similar to those known to occur in certain other virus diseases. The infectivity of the virus is greater for the fetus than for the postnatal guinea pig. Natural passive immunization in utero or per colostrum is inadequate to protect fetuses or suckling guinea pigs against experimental infection. In spontaneously infected adults the quality or duration of active immunity may depend on the presence of active lesions. An analogy between the distribution of the inclusion bodies sometimes found in stillborn and premature human infants and the distribution of such bodies in experimentally infected guinea pig fetuses is pointed out.

FROM AUTHOR'S SUMMARY.

EFFECT OF FORMALDEHYDE ON PNEUMOCOCCI. R. J. DUBOS, J. Exper. Med. **67**: 389, 1938.

When used in low concentration, formaldehyde increases the rate of autolytic disintegration of pneumococci, whereas in high concentration it completely inhibits autolysis and preserves both the morphologic and the staining characteristics of the cells. Pneumococci treated with formaldehyde in high concentration, then washed free from the antiseptic and resuspended in physiologic solution of sodium chloride rapidly undergo a change which renders them gram-negative and smaller.

The lysis is only partial, however, and is not accompanied by disintegration of the cell. It is caused by the autolytic enzyme of the cell, which remains inactive in the presence of an excess of formaldehyde but recovers its activity when the cells are resuspended in a neutral medium after removal of the antiseptic. If the autolytic enzyme is irreversibly inactivated by heating, or if it is maintained inactive in an acid or an alkaline reaction, the formaldehydized cells retain their staining characteristics and morphologic integrity. Formaldehydized pneumococci which have become gram-negative owing to the action of their autolytic enzyme fail to elicit antibodies for the type-specific carbohydrate when injected into rabbits. Formaldehydized pneumococci in which the autolytic enzyme has been destroyed or maintained inactive and which have retained their gram-positive character function as a very effective type-specific antigen in the rabbit. These observations emphasize once more the close relation between the gram-positive structure of pneumococci and the capsular polysaccharide antigen of the cell. They can be used as a basis for the preparation of suspensions of formaldehydized pneumococci which will be stable and effective as type-specific antigens.

FROM AUTHOR'S SUMMARY.

INHIBITORY SUBSTANCE FOR INFLUENZA ORGANISMS. E. KRUMWIEDE and A. G. KUTTNER, *J. Exper. Med.* **67**:429, 1938.

Five per cent sheep blood agar is a selective medium for beta hemolytic streptococci in throat cultures since sheep blood inhibits the growth of bacillus X (*Haemophilus haemolyticus*) and *Bacillus parainfluenzae haemolyticus*. The growth of *Haemophilus influenzae* is also inhibited by sheep blood. This inhibitory action resides in the erythrocytes and is thermolabile. The inhibitor is not affected by disruption of the erythrocytes in laking. A similar inhibitory action on the growth of hemolytic and nonhemolytic members of the influenza group is noted with blood from animals closely related to the sheep, such as the goat and the cow; human blood contains a similar but less powerful inhibitor. Members of the influenza group grow well on unheated rodent blood: rabbit, guinea pig and rat. These organisms also grow fairly well on unheated horse blood.

FROM AUTHORS' SUMMARY.

EFFECTS OF ACIDITY ON PNEUMOCOCCUS GROWTH. W. H. KELLEY, *J. Exper. Med.* **67**:667, 1938.

In the presence of animal fluids or their protein constituents, type I pneumococci survived and multiplied at acid hydrogen ion concentrations which in the plain broth were bactericidal for these organisms. Minimal numbers of these cells readily produced growth in serum broth when the broth was adjusted at a hydrogen ion concentration as great as p_H 5.5 with hydrochloric acid or to p_H 6.5 with acetic acid. Growth of the pneumococci could be demonstrated in serum broth adjusted to p_H 5 with hydrochloric acid or to p_H 5.5 with acetic acid, although at these hydrogen ion concentrations large amounts of inoculum were necessary. Similar results were obtained with broth to which certain animal proteins had been added and in serum broth which had been heated in the autoclave at 20 pounds' (9 Kg.) pressure for twenty minutes. *Pneumococcus* growth proceeded at a more rapid rate in serum-dextrose broth at p_H 6.5 than in dextrose broth at the optimal hydrogen ion concentration of p_H 7.8. At p_H 6 large numbers of pneumococci failed to produce the same amount of growth in serum-dextrose broth as at p_H 6.5 or in dextrose broth as at p_H 7.8. It is of interest that in cultures in serum-dextrose broth the stationary and decline phases of pneumococcus growth were prolonged, and cell death delayed, in comparison with cultures in dextrose broth alone.

FROM AUTHOR'S SUMMARY.

SATELLITE HEMOLYTIC ZONES IN BLOOD AGAR STAPHYLOCOCCUS CULTURES. G. B. RHODES, *J. Infect. Dis.* **62**:124, 1938.

From certain strains of growing hemolytic *Staphylococcus aureus* a substance is diffused which produces discrete hemolytic zones in blood agar plates. These occur only when unheated serum is in the medium and are unrelated to the complement content of such serum. The occurrence, and the number and size of the zones vary with the serum and erythrocytes of different animals.

FROM AUTHOR'S SUMMARY.

EXPERIMENTAL INVESTIGATIONS IN HEMORRHAGIC ENCEPHALITIS. A. B. BAKER and C. W. BUGGS, *J. Infect. Dis.* **62**:293, 1938.

Fresh brain tissue obtained from a person who died of hemorrhagic encephalitis and injected intracerebrally into experimental animals proved very infectious to rabbits. Guinea pigs and white mice did not react to it. The active agent was passed serially through sixteen sets of animals before it was lost. All the animals presented the characteristic symptoms of an involvement of the central nervous system: hyperirritability, muscular twitchings, motor weakness, convulsions and moderate salivation. The active agent lost its potency when stored in 50 per cent glycerol at 0 C. for one month. Attempts to pass it through a filter proved unsuccessful. One instance is noted in which it proved infectious via subcutaneous inoculation. The brains of the inoculated rabbits revealed changes quite similar to those found in man in hemorrhagic encephalitis.

FROM AUTHORS' SUMMARY.

OBSERVATIONS ON LIVING VACCINIA AND ECTROMELIA VIRUSES BY HIGH POWER MICROSCOPY. F. HIMMELWEIT, *Brit. J. Exper. Path.* **19**:108, 1938.

A method is described by which the living chorioallantoic membrane may be examined microscopically by annular oblique incident illumination while it is still in situ in the egg, with its vascular and other anatomic connections still undisturbed. The method has been applied to the study of virus bodies within the living cells of the chorioallantoic membrane of the duck egg infected with ectromelia and vaccinia viruses. Observations on the nature of the ectromelia inclusion body are reported, and the formation of extracellular giant aggregates is described. The presence and distribution of vaccinal elementary bodies within the living cell have been observed from early stages of cell infection. The elementary bodies as they exist in the cell are contained within a matrix of low viscosity, and their partial release in "extrusion bodies" is described. It is concluded that the vaccinal elementary body represents the only virus structure which can be recognized in the living cell and that the Guarnieri bodies seen in stained preparations correspond simply to localized irregular collections of elementary bodies and do not necessarily represent all the virus in the cell.

FROM AUTHOR'S SUMMARY.

TUBERCULOSIS IN AMERICAN SCHOOLS AND COLLEGES. E. R. LONG, *Tubercle* **19**:241, 1938.

Studies continued over a decade indicate that the proportion of elementary and high school children positive to the tuberculin test is steadily falling. In the less crowded communities the drop is more striking than in large, congested cities. The incidence of the positive result is generally significantly lower in rural communities than in urban areas, and differences within the same community are always found corresponding to differences in economic level. Important tuberculous disease is first encountered to an appreciable extent in high school students, in whom the combined incidence of latent and manifest disease varies from 1.5 to nearly 3 per cent, being higher in girls. Important disease demanding care occurs in from 0.5 to 1 per cent of these children. Active programs for the

early detection and control of tuberculosis in schools now operate throughout the country. The basic principles of these programs are mass tuberculin testing, roentgen examination of the positive reactors and provision of suitable care for those found to have lesions. In the colleges about 6 students per thousand have tuberculosis of the adult type. A marked variation occurs geographically, students from the great central portion of the country having a relatively low incidence of the disease. Tuberculosis is recognized as the most serious disease of the college period yet as insidious in onset, requiring routine mass measures for its detection. Most of the lesions now discovered are in the minimal stage. Students of medicine and nursing are known to be subject to a special hazard. The danger for medical students seems greatest during the third and fourth, or clinical, years, suggesting that tuberculosis acquired in the medical school is usually exogenous. Most nurses who are negative to tuberculin on beginning training become reactors during their course. The incidence of clinical tuberculosis is also proportionately high in nurses as compared with other professional or working groups at the same age, both during the period of training and in the first years after qualification.

POLYMORPHISM OF RICKETTSIAS OF TRACHOMA. A CUÉNOD and R. NATAF, Arch. Inst. Pasteur de Tunis **27**:1, 1938.

Rickettsias of trachoma vary from extremely minute forms to cells as large as from 1 to 3 microns. The larger forms are visible in unstained trachomatous material under the high-dry objective. They are highly refractile and appear like brilliant luminous pearls which may be spheroid, ellipsoid or rarely rhomboid. They occur in groups of from 12 to 15, encircling the nuclei of epithelial cells. Extracellularly they are chiefly in pairs resembling dumbbells or thick rods. These rickettsias stain reddish purple by the Giemsa method but do not stain with ordinary aniline dyes. These forms are found also in inoculated lice, guinea pigs and rabbits.

J. B. GUNNISON.

EXPERIMENTAL STUDIES OF HERPES VIRUS IN WHITE MICE. E. GILDEMEISTER and I. AHLFELD, Zentralbl. f. Bakt. (Abt. 1) **139**:325, 1937.

Gildemeister and Ahlfeld were unable to infect white mice orally or monkeys cutaneously with herpes virus (strain Basel III). Normal rabbit serum contained no protective antibodies against cutaneous infection of white mice with herpes virus. Active immunization of rabbits with this virus, however, led to development of neutralizing antibodies. The serum of many persons also contained protective antibodies against herpes virus, not only that from persons who had often suffered from herpes but also that from persons who had not had herpes. White mice could be immunized passively with rabbit antiherpes serum, but the immunity lasted only a few days. Such serum also had therapeutic value in mice suffering from herpes. The immunity of white mice following herpetic infection was of short duration.

PAUL R. CANNON.

BACILLUS VAGINALIS OF DÖDERLEIN AS A CAUSE OF ENDOCARDITIS. FRED MARSHALL, Zentralbl. f. Bakt. (Abt. 1) **141**:153, 1938.

A case of ulcerative endocarditis in a woman 21 years of age is described, in which a pure culture of acidophilic bacilli was obtained from the blood before death and another from the mitral valve after death. This micro-organism was identified by cultural and serologic tests as *Bacillus vaginalis* of Döderlein. It is considered by the author to have been the cause of the endocarditis because of the finding of the organism in a blood culture before death, the isolation of the organism in pure culture from several organs after death, the presence of agglutinins to the organism in the blood serum and the demonstration of the bacilli in the endocarditic lesions.

PAUL R. CANNON.

Immunology

INTRACUTANEOUS REACTIONS AGAINST ANTISERUM IN TUBERCULOSIS. H. J. CORPER and C. B. VIDAL, *Am. Rev. Tuberc.* **37**:239, 1938.

Although it is conceded on the basis of prior experiments that there is a specific immunity to tuberculosis, demonstrable both in man and animals, it is found impossible to demonstrate the presence of such immunity by means of intracutaneous tests with specific antisera. All attempts to produce an antiserum that would react specifically on intracutaneous injection into animals of the same species (in order to exclude foreign serum reactions) proved futile. Antisera were prepared in guinea pigs, rabbits and dogs and were injected into animals infected with virulent and avirulent human tubercle bacilli. In human subjects, both normal and tuberculous, with negative and positive reactions to tuberculin (purified protein derivative), no specific reactions were obtained to antiserum. Serum from normal and from immunized goats produced intracutaneous reactions in normal and tuberculous human subjects. These reactions developed after from three days to two weeks and persisted for from several days to a week; they were of variable intensity.

H. J. CORPER.

IMMUNIZING SUBSTANCES IN PNEUMOCOCCI. L. D. FELTON and G. KAUFFMANN, *Bull. Johns Hopkins Hosp.* **62**:430, 1938.

The results of this study indicate that the amount of antigenic substance in the bacterial cells varies with growth. Young cultures demonstrated high antigenicity both of the bacterial cell and of the substance extracted from it; from old cultures, of low antigenicity, only a relatively small yield of active substance was obtained. The "essential immunizing antigen" of the pneumococcus has been defined as that substance isolated either from the cell or from the culture medium which contains as many immunizing doses for mice as the bacterial cells or fluid from which it is derived. Felton and Kauffmann have demonstrated the possibility that from pneumococci of types I and II fractions may be isolated which contain from four to eleven hundred times as many immunizing doses as do the original cells from which the fractions were made. This degree of activity is demonstrable, at least in white mice, by extracting directly from the cells the fraction soluble at pH 3 in hydrochloric acid or in 5 per cent trichloroacetic acid or by extracting the fraction after digestion of the cells with trypsin, pancreatin or papain. The weight of dried organisms of type I containing a million immunizing doses varied from 1 to 10,000 Gm., whereas the weight of the isolated fraction containing the same number of immunizing doses varied from 0.012 to 5 Gm. With organisms of type II, the corresponding weights were from 0.8 to 100 Gm. and from 0.01 to 0.5 Gm. At least 85 per cent of the bacterial cell was found to be inert. The chemical nature of this fraction was not entered into except for the dextrose number. The amount of this component did not correlate with the degree of antigenic activity. The authors describe an experiment with organisms of type I and another with organisms of type II in which after heating the organisms in hundredth-normal alkali at 100 C. for thirty minutes an alcohol-soluble fraction was obtained which was as active as any other fraction of the entire cell so far isolated.

FROM AUTHORS' SUMMARY.

BLOOD-GROUPING AND COMPATIBILITY. P. HOXWORTH and A. AMES, *J. A. M. A.* **108**:1234, 1937.

Hoxworth and Ames employed a modification of the technics of Vincent and Coca for the determination of blood grouping and compatibility. In the determination of grouping large drops of high-titered test serums, anti-B and anti-A were added to the left and right ends, respectively, of a glass slide. Blood obtained by puncture of a finger and defibrinated by whipping was added to each drop of

serum and mixed by means of a platinum loop. In matching bloods a drop of a 50 per cent suspension of the recipient's defibrinated blood in saline solution was mixed with one fifth of a drop of 50 per cent suspension of the donor's defibrinated blood. The mixture was agitated and observed after fifteen minutes. This method was employed for more than 400 transfusions, without a reaction. Agglutination was prompt and clearly defined, and the readings were in agreement with those determined by the older methods. This method obviated the need for venipuncture and for separation of cells and serum. The 1:5 mixture of the donor's and the recipient's blood was needed for the detection of universal donors with dangerous amounts of agglutinin for A or B recipients. FREDERICK STENN.

AN IMPROVED AIR DRIVEN TYPE OF ULTRACENTRIFUGE FOR MOLECULAR SEDIMENTATION. J. H. BAUER and E. G. PICKELS, *J. Exper. Med.* **65**:565, 1937.

A description is given of the construction and operation of an improved type of air-driven ultracentrifuge, operating in a vacuum and suitable for the determination of sedimentation constants of protein molecules. The rotor of the centrifuge is made of a forged aluminum alloy; it is oval, measures 185 mm. at its greatest diameter and weighs 3,430 Gm. It carries a transparent cell located at a distance of 65 mm. from the axis of rotation and designed to accommodate a fluid column 15 mm. high. The rotor has been run repeatedly over long periods at a speed of 60,000 revolutions per minute, which corresponds to a centrifugal force of 260,000 times gravity in the center of the cell. At this speed no deformation of the rotor or leakage of the cell has been observed. The sharp definition of sedimentation photographs taken at high speed serves to indicate the absence of detectable vibrations in the centrifuge. When a vacuum of less than 1 micron of mercury is maintained in the centrifuge chamber, the rise in the temperature of the rotor amounts to only 1 or 2 C. after several hours' run at high speed. There has been no evidence of convection currents interfering with normal sedimentation of protein molecules in the centrifugal field. A driving air pressure of about 18 pounds per square inch (8 Kg. to 6 sq. cm.) is sufficient to maintain the centrifuge at a steady speed of 60,000 revolutions per minute. With a driving pressure of 80 pounds per square inch (36 Kg. to 6 sq. cm.) it can be accelerated to this speed in less than twenty minutes, and it may also be brought to rest in about the same length of time by the application of the braking system. The adaptation of Svedberg's optical systems to this centrifuge for photographically recording the movement of sedimentation boundaries is described.

FROM THE AUTHORS' SUMMARY.

REACTIONS OF ANTI-AZOPROTEINS SERUM. K. LANDSTEINER and J. VAN DER SCHEER, *J. Exper. Med.* **67**:709, 1938.

Azoproteins have been prepared with azocomponents possessing two serologically active groups. On immunization with such antigens immune serums were obtained containing two separate, unrelated antibodies, each specific for one of the two groups and separable by absorption. In other cases one of the two structures was dominant in that antibodies were formed only toward this and not toward the other grouping. The specificity of the antibodies was in general found to be influenced to some extent by the presence of a second group in the antigen. The relevancy of these observations for antibodies directed against natural antigens has been noted.

FROM AUTHORS' SUMMARY.

STATISTICAL STUDIES OF VACCINE VIRUS. R. F. PARKER, *J. Exper. Med.* **67**:725, 1938.

A method has been described by which it is possible to estimate the number of particles of vaccine virus which are required to cause infection in the skin of a rabbit. The method consists essentially in injecting suitably diluted suspensions

of the virus intradermally into rabbits in series. The percentage of inoculations at each dilution giving rise to lesions was observed, and the data are subjected to appropriate statistical analysis. Several strains of vaccine virus, differing in their characteristics, have been studied, with the following results: A single particle of the virus prepared by the New York City Board of Health appears to give infection or inoculation. The same is true for the strain derived from it but cultured in a chick embryo-Tyrode solution medium for a prolonged period. This strain, as has been noted, has largely lost its ability to cause extensive necrosis in the rabbit's skin and causes generalized infection only exceptionally. From the results reported here it appears that the alteration in the character of the lesion must be traced to factors other than the reduced ability of the virus to establish a foothold in the animal organisms. In this respect the cultured virus appears to be the equal of the original passage virus. Similarly the Noguchi strain of virus is apparently capable of infecting when a single particle is properly introduced.

FROM AUTHOR'S SUMMARY.

IN VITRO ACTION OF IMMUNE RAT SERUM ON THE NEMATODE, NIPPOSTRONGYLUS MURIS. M. P. SARLES, J. Infect. Dis. 62:337, 1938.

Preparasitic infective larvae were freed from bacteria adherent to the cuticula by treatment with a 0.1 per cent solution of mercuric chloride, were sealed with petrolatum between a sterile slide and a cover slip in small drops of serum or saline solution, were kept at room temperature and at 36 C. and were observed at frequent intervals with regard to the occurrence of precipitates and their development and survival. This test was made on 14 immune rat serums, 13 normal rat serums and saline solution. The immune serums were from rats repeatedly infected and had been proved to have an antibody content by their power to immunize normal rats passively. Also tested were parasites in the pulmonary and intestinal stages from rats, in 4 immune and 4 normal serums and in saline solution. In all 3 stages the parasites survived longer and were more active in serum than in saline solution; they survived about an equal time in normal and immune serums and in both were seen actively feeding by vigorous rhythmic contractions of the esophagus. Only infective larvae developed in vitro, passing through what corresponded to the parasitic phase of development in the skin, but their development was less marked in immune than in normal serum. Evidences of antibody action, seen with worms in immune serums but not with those in normal serums or saline solution, included: (1) invariable formation of precipitates of (a) cuticular type (with larvae and parasites in the pulmonary stage only), (b) excretory type, (c) oral type and (d) intestinal type and (2) sometimes decreased activity (of larvae and parasites in the pulmonary stage) and inhibition of development (of larvae). The correspondence of certain of these reactions to those seen in actively and passively immunized rats and their probable role in acquired immunity to Nippostrongylus muris are discussed.

FROM AUTHOR'S SUMMARY.

THE ISOLATION OF ANTIGENIC SUBSTANCES FROM STRAINS OF BACTERIUM TYPHOSUM. D. W. HENDERSON and W. T. J. MORGAN, Brit. J. Exper. Path. 19:82, 1938.

When suitable strains of *Bacterium typhosum* are extracted with anhydrous diethylene glycol, substances are obtained which are apparently free from protein and which are antigenically active. In extracts from rough Vi strains Vi antigen can be detected as a chemical entity separate and distinct from O substance. From O agglutinable strains substances containing O antigen are readily isolated, but the authors have not succeeded in obtaining these preparations entirely free from Vi antigen. In extracts obtained from strains that are rich in flagella traces of H antigen have been detected. This finding was probably due to passage of flagellar

débris through the bacterial filters used in the preparation of the extracts. The process of extraction modifies the functional activity of the antigenic complex in rough Vi strains: Immunization with these extracts produces an immune body that is much less effective in protecting experimental animals than that produced by immunizing with living suspensions of rough Vi bacilli. The value of mucin as an adjuvant to the test dose of typhoid bacilli for experiments on typhoid infection in mice has been confirmed.

FROM AUTHORS' SUMMARY.

THE FORMAMIDE METHOD FOR THE EXTRACTION OF POLYSACCHARIDES FROM HEMOLYTIC STREPTOCOCCI. A. T. FULLER, *Brit. J. Exper. Path.* **19**:130, 1938.

The formamide method for preparing group-specific extracts from hemolytic streptococci is described. It has the following advantages over existing methods: 1. It completely dissolves the bacteria, thereby giving potent extracts. 2. It destroys or removes protein substances that might give cross reactions.

A preliminary purification of the group-specific polysaccharides is described. It is suggested that the method may be applicable to all species of bacteria.

FROM AUTHOR'S SUMMARY.

THE PREPARATION OF SERUM PROTECTIVE AGAINST HEMOLYTIC STREPTOCOCCI. H. LOEWENTHAL, *Brit. J. Exper. Path.* **19**:143, 1938.

The antigen responsible for the production of the antibody effective against the invasiveness of hemolytic streptococci is intimately associated with the capsule which develops on these cocci in the early hours of growth in culture. This antigen is extremely labile, being readily destroyed by heat and by low concentrations of formaldehyde or of merthiolate. A temperature of 55 C. applied to a suspension of young encapsulated cocci for twelve minutes kills the organisms but leaves the capsular antigen intact. More prolonged application of this temperature rapidly destroys this antigen. With suspensions of young cultures which have been cautiously killed with heat, it has been possible for the first time to prepare a serum potentially protective against hemolytic streptococci in the mucoid phase. This method of immunization also gives rise, more rapidly and regularly than do the old methods, to antibody protective against nonmucoid strains. The result of a single experiment suggests that it is possible to produce a potent polyvalent serum by immunizing with a number of strains simultaneously.

FROM AUTHOR'S SUMMARY.

RELATION OF THE COLLOIDAL STRUCTURE TO THE ACTION OF COMPLEMENT AND TO PAROXYSMAL HEMOGLOBINURIA. H. SACHS, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **91**:328, 1937.

The ability of complement to lyse red blood cells on addition of silicic acid, snake venom, insulin or tannic acid in the absence of a hemolytic antibody suggests that the latter is not absolutely essential for the lytic action of complement. The supposition is made that the aforementioned substances substitute for the specific antibody. The observation that in a hypotonic solution complement alone may cause lysis forces one to conclude that colloidal phenomena and not substitution of nonspecific substances for the lytic antibody are at play. How can this be explained? Sachs suggests that the phenomenon is essentially the same as in the true antigen-antibody reaction. Here the globulin of the antibody forms a film on the surface of the antigen, and the action of the complement follows. The lysis without the lytic antibody can be explained as due to alteration of the globulin of the complement by the mentioned substances or by the hypotonicity of the medium. This concept makes it possible to explain paroxysmal hemoglobinuria without assumption of a hypothetical autoantibody. In this instance, chilling would be the cause of the alteration of the globulins in the patient's serum, and lysis would take place after the chilling was over.

I. DAVIDSOHN.

IMMUNITY AGAINST PYOGENIC STAPHYLOCOCCI. A. PETTERSSON, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **91**:366, 1937.

The fatal dose when staphylococci were injected intravenously was from forty to one hundred times smaller than the dose by any other route. In the defense against staphylococci, leukocytes play the main part, as shown by the protective influence exerted by them when mixtures of leukocytes and staphylococci were injected into rabbits. The serum of animals inoculated with staphylococci did not inhibit leukocytic phagocytosis to any greater extent than normal serum did, but the leukocytes of immunized animals had greater phagocytic ability than the leukocytes of nonimmunized animals. Staphylococci induce negative chemotaxis in leukocytes, which keeps the latter away. That action is responsible for the edema and interferes with the formation of abscesses. Pettersson advocates that in the preparation of immune serums attention be paid to the formation of antibodies against the negative chemical influence of staphylococci.

I. DAVIDSOHN.

ELIMINATION OF GROUP SPECIFIC SUBSTANCES. P. DAHR and H. LINDAU, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **91**:470, 1937.

The substance A was detected in the saliva and urine of a chimpanzee and in the urine but not in the saliva of another chimpanzee; both apes belonged to group A. This report brings the number of grouped chimpanzees to 90, 78 of whom were classified as A and 12 as O. The group property B was shown to consist of three fractions— B_1 , B_2 and B_3 . The B_2 and B_3 fractions were found in various animal species, the B_1 only in man and in anthropoid apes. The distribution of the fractions B_2 and B_3 in animals is not always identical in the red blood cells and in the different secretions; for instance, in one rabbit the red blood cells had the fractions B_2 and B_3 and the saliva only B_3 . In some monkeys the blood had only one fraction and the saliva two. The discrepancies in reports due to elimination of the property B in secretions on one occasion and the failure to find it on other occasions may be explained by the use of testing serums with different anti-B fractions.

I. DAVIDSOHN.

ISOAGGLUTININ ANTI-M. V. FRIEDENREICH, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **91**:485, 1937.

The blood serum of a 2½ month old baby whose blood was of group ON was found to contain the isoagglutinin anti-M. The mother's blood belonged to the same group but had no such isoagglutinin. As would be expected, the serum of the infant did not have anti-A and anti-B isoagglutinins. This serum containing the unusual anti-M agglutinin was equal in titer to the usual immune serums and reacted similarly in absorption experiments. The examination was repeated after one week with the same results. The father and 3 siblings of the mother were examined, with negative results. The observation was made in the course of litigation over the alleged paternity. Three men who were considered as possible fathers did not have the isoagglutinin anti-M in their blood. The child died at the age of 8 months, which prevented further investigation.

I. DAVIDSOHN.

DISTRIBUTION OF THE BLOOD GROUPS IN FINNS. U. P. KOKKO, *Acta Soc. med. fenn. duodecim* (Ser. A, no. 3, art. 14) **19**:1, 1937.

Blood specimens to the number of 1,518 were grouped by testing both cells and serum. Bloods from 1,334 Finnish-speaking persons in this series were distributed among the blood groups as follows: group O, 32.5 per cent; group A, 42.2 per cent; group B, 17.5 per cent, and group AB, 7.8 per cent. This agrees closely with previously published findings.

A. S. WIENER.

Tumors

EFFECT OF OIL OF WINTERGREEN ON SPONTANEOUS TUMORS OF THE MAMMARY GLANDS IN MICE. L. C. STRONG, *Am. J. Cancer* **32**:227, 1938.

Two fractions obtained by distillation of the true oil of wintergreen have different effects on spontaneous tumors of the mammary glands of mice. The high fraction has no effect on (1) the rate of growth of the tumors, (2) the time of survival of the animals or (3) the histologic structure of the tumors. The low fraction has a pronounced effect, causing (1) slowing of the rate of growth of the tumors, with complete regression in 4 of 34 animals, (2) increase in the time of survival after the onset of cancer and (3) gross and histologic alterations in the tumors. The action of the low fraction appears to be more pronounced than the action of the true oil of wintergreen. The difference in effect of the two fractions depends on the chemical difference between them and not on the methyl salicylate content which they have in common.

FROM AUTHOR'S CONCLUSIONS.

IMMUNIZATION AGAINST NEOPLASM: ITS EFFECT ON THE NITROGEN METABOLISM OF THE HOST. R. H. OSTER and W. T. SALTER, *Am. J. Cancer* **32**:422, 1938.

In 1925 Dodds found that certain rats which resisted inoculation with neoplasms showed a low concentration of urea in the blood after treatment with roentgen rays. Independently several investigators had shown that inoculation of tumors into mice may confer immunity to subsequent inoculation, an immunity which persists after removal of the tumors. This paper presents evidence that animals so immunized show the peculiarity in nitrogen metabolism which Dodds described in naturally immune animals. It suggests that susceptibility to malignant disease has a definite chemical background. The apparent concentration of urea in the blood of normal and tumor-bearing mice at the end of a period of fasting (twenty-four hours) is about 30 mg. per hundred cubic centimeters even after roentgen irradiation. Immune animals show the same value for urea until irradiated. Thereupon the apparent blood urea drops steadily for three days, to about 20 mg. per hundred cubic centimeters, and climbs back to normal in the course of the next week. The excretion of nitrogen in the urine shows urea to be consistently about 81 per cent and ammonia 9 per cent of the total nitrogen excreted on a mixed diet. This is true despite an excessive excretion of nitrogen during the day after roentgen treatment in both normal and immune animals. The drop in blood urea, therefore, is not due to a specific failure of the normal urea-producing mechanism. The response of apparent blood urea is independent of the actual presence of a tumor and indicates that the "immunity" is a property of the host primarily. Indeed, under certain special laboratory conditions, the chemical reaction may be used statistically to predict in average figures the approximate fate of inoculated neoplasms subsequently introduced. These observations suggest that the difference between a malignant and a benign tumor may reside in part in the chemical constitution of the host.

FROM AUTHORS' SUMMARY.

TUMOR GROWTH IN MICE ONE-FIFTH SATURATED WITH DEUTERIUM OXIDE (HEAVY WATER). H. G. BARBOUR and E. ALLEN, *Am. J. Cancer* **32**:440, 1938.

The growth of carcinoma in 7 mice and of lymphosarcoma in 2 mice was half as rapid, or less, in mice drinking 40 per cent heavy water (deuterium oxide) as in mice drinking ordinary water. Drinking 40 per cent deuterium oxide did not decrease the weight of young adult mice given injections of carcinoma or of lymphosarcoma. Mice receiving ordinary water grew faster, but much of the difference in weight could be accounted for by their larger tumors. Mice receiving deuterium oxide drank less than their controls receiving ordinary water by about a third or more. Survival of the tumor-bearing mice was shortened by deuterium oxide. Carcinomatous mice drinking 60 per cent heavy water failed to maintain

their body weight and died sooner than those receiving 40 per cent heavy water. It is not yet certain whether deuterium oxide exerts a specific action on the tumors in question by virtue of its osmotic properties or by virtue of its capacity to interfere with enzyme systems or whether the tumors are influenced by a growth-inhibiting property or merely by established catabolic effect of deuterium oxide.

FROM AUTHORS' SUMMARY.

AGENT AND SOIL IN EXPERIMENTAL CARCINOGENESIS. W. H. WOGLOM, *Am. J. Cancer* **32**:447, 1938.

In the rat, relatively gross trauma does not lower the resistance of glandular epithelium to benzpyrene.

FROM AUTHOR'S CONCLUSION.

THE DEVELOPMENT OF SARCOMA IN MICE INJECTED WITH HORMONES OR HORMONE-LIKE SUBSTANCES. E. L. BURNS, V. SUNTZEFF and L. LOEB, *Am. J. Cancer* **32**:534, 1938.

Among 247 mice which had received injections of various endocrine preparations, including an extract of liver—most of them over relatively long periods of time—10 showed development of sarcoma. In 128 control mice, likewise observed over long periods, sarcoma did not appear. In 9 of the sarcoma-bearing mice ordinary spindle-cell sarcoma was formed; in 1 the rapidly growing tumor originated probably in striated muscle tissue. All the tumors except 1 developed at or near places where the tissues had been stimulated by the injections. Specificity of the stimulating factors is less pronounced in the case of sarcoma than in the case of epithelial proliferation induced by an estrogen, but the exact nature of the stimulating agent still needs to be determined. These tumors developed only after injections over a long period. While graded growth processes leading step by step toward cancerous growth can be recognized preceding the formation of epithelial tumors following injections of estrogenic substances, the growth of sarcomatous tumors seems to start suddenly. It is possible, however, that here, also, transitional growth processes precede the development of malignant growth.

FROM AUTHORS' SUMMARY.

A TRANSPLANTABLE UTERINE RAT SARCOMA OF 100 PER CENT TRANSMISSIBILITY. J. A. POLLIA, *Am. J. Cancer* **32**:545, 1938.

A new transplantable uterine sarcoma of 100 per cent transmissibility in the pure strain of animals from which it originated is described. In other standard strains the transmissibility is only about 20 per cent. The consistency with which the transplant "takes," the rapidity of growth and the inability to stop its development with any procedure other than irradiation make it a favorable medium for the study of the effect of alleged therapeutic agents on transplantable animal tumors.

FROM AUTHOR'S SUMMARY.

ROLE OF THE NEURAL CRESTS IN THE EMBRYONAL ADENOSARCOMAS OF THE KIDNEY. P. MASON, *Am. J. Cancer* **33**:1, 1938.

The three renal tumors studied in this work present the classic characteristics of embryonal adenosarcoma. Study of the tumors by ordinary and by neurologic methods shows that they are composed not only of the renal glandular elements, mesenchymatous elements and striated muscle admitted by the majority of authors but also of neuroepithelial and nerve elements which can be linked with the lateral lumbar neuroepithelium and the neural crests derived from it. Their "nephrogenous blastema" is composed of undifferentiated cellular cords formed by neuroepithelial vesicles. These cords have the structure of neural crests and give origin first to neuroblasts, especially of a sympathetic type, to lemmoblasts, to a mesodermal mesenchyme and to striated muscle fibers. These same cords later dis-

appear in giving birth to rudimentary nephrons. The tumor blastema is, therefore, neurogenic, sclerogenic, myogenic and nephrogenic. Its neurogenic properties show the certain participation of the lumbolateral neuroepithelium and its crests in the construction of mixed tumors of the kidney. Its myogenic and nephrogenic properties raise a question: Are the lumbar crests the possible source of certain striated muscles and of the metanephrogenic blastema? The reply belongs to experimental embryology. If one reflects on the frequent presence of spinal and sympathetic ganglions in a number of teratomas composed of adult tissue, it is permissible to believe that these elements themselves come from neural crests but that these neural crests have existed as such only in the very earliest stages of the development of the tumors and have rapidly disappeared as they do in the normal embryo because they have become differentiated. It is only in malignant teratomas with immature tissues that one can hope to encounter them.

FROM AUTHOR'S SUMMARY.

"INFLAMMATORY CARCINOMA" OF THE BREAST. G. W. TAYLOR and A. MELTZER, *Am. J. Cancer* **33**:33, 1938.

A clinical study was made of 38 cases of inflammatory carcinoma of the breast encountered over a nine and one-half year period. This grave disease is not rare. The literature contains records of over a hundred cases. In the present series the incidence was 4 per cent of the total number of cancers of the breast. Although the disease is rare after the age of 70, its distribution with respect to age is the same as for cancer of the breast in general. The inflammatory signs may arise simultaneously with the cancer (primary type), or they may occur after a scirrhus cancer has been present for some time (secondary type). In the primary group a common early symptom is pain. The signs of inflammation may lead to a mistaken diagnosis and injudicious early therapy. The primary cancer of the breast comes to medical attention early, yet on admission the signs of inflammation are usually full blown and the disease widespread. The cancer may have an acute erysipeloid distribution, or it may show a tendency to nodular localization. It rarely ulcerates. It spreads rapidly in the superficial lymphatic structures of the thoracic wall. Multiple visceral metastases occur early, but the rapid course of the disease often does not permit them to attain clinical recognition. Metastases in bones were recognized roentgenologically in only 4 cases of the primary type. In the uncomplicated cases leukocytosis, fever and other signs of toxicity are rare. The patients maintain remarkably good health through the greater part of the course, and cachexia is unusual. Death is most often due to intrathoracic complications. The average duration of life in cases of the primary type was 21.3 months; in cases of the secondary type it was 10.8 months after the appearance of inflammatory signs. The inflammatory signs—edema, redness and heat—are due to extensive blockage of lymphatic channels by the cancer and to congestion of the subpapillary plexus. There is no uniform pathologic type. The large fatty breast seems predisposed, as does the hyperplastic breast of the woman whose pregnancy is far advanced or of the lactating mother. No other predisposing factors could be established. The results of therapy are poor. Surgical removal is followed by prompt evidence of supraclavicular disease, recurrence in the skin or invasion of the opposite breast. Roentgen therapy seems to give the best palliative results. Artificial menopause does not alter the course of the disease.

FROM AUTHORS' SUMMARY.

SPONTANEOUS BONE TUMORS OF MICE. F. C. PYBUS and E. W. WHITE, *Am. J. Cancer* **33**:98, 1938.

From 2 mice of Simpson strain 3 has been derived a selected inbred branch in which the incidence of bone tumors (sarcoma) is very high. A brief account is given of the main types and sites of these tumors and of their distribution with respect to age.

FROM AUTHORS' SUMMARY.

A TRANSMISSIBLE LEUKEMIA IN THE "A" STRAIN OF MICE. J. H. LAWRENCE and W. U. GARDNER, *Am. J. Cancer* **33**:112, 1938.

Transmissible lymphatic leukemia has occurred in the Strong A strain of mice. It arose in a mouse receiving a prolonged course of injections of an estrogenic substance and has been transferred by subcutaneous and by intravenous injections of suspensions of minced spleen or lymph nodes, invariably giving 100 per cent "takes" and regularly causing death of the animals. The disease could not be produced by intravenous inoculation of cell-free filtrates. It could not be transmitted to mice of another strain.

FROM AUTHORS' SUMMARY.

NESIDIOBLASTOMA, THE ISLET TUMOR OF THE PANCREAS. G. F. LAIDLAW, *Am. J. Path.* **14**:125, 1938.

Microscopically, the chief feature of nesidioblastoma in most of the instances is the exact duplication of the pattern of normal islets. The growths also resemble hypertrophied islets in their tendency to exaggerate some features of the normal islet pattern. Just as the tumors duplicate the structure of normal and hypertrophied islets, so they are subject to the same pathologic vicissitudes, such as fibrosis, hyaline degeneration and calcification. The origin of the tumor cells is indicated by the abundance of figures showing the epithelial lining of the duct continuous with a group of tumor cells. The origin of the name "nesidioblastoma" is explained.

FROM AUTHOR'S SUMMARY.

TRANSMISSION OF CHLOROLEUKEMIA OF MICE. J. W. HALL and F. J. KNOCKE, *Am. J. Path.* **14**:217, 1938.

A strain of chloroleukemia of mice is described that was readily transmitted to related mice by intravenous injections of a suspension of the leukemic cells. Subcutaneous inoculation of the leukemic leukocytes produced localized tumors at the sites of inoculation in approximately 23 per cent of the inoculated mice. These tumors grew slowly. Intravenous injection of a suspension of the leukemic cells produced rapidly progressing generalized leukemia, fatal after approximately twenty days in 95.1 per cent of the mice receiving the cells. This observation indicates that large numbers of leukemic cells were destroyed in the subcutaneous tissues of mice that were susceptible to intravenous administration of similar cells. Suspensions of tumor cells injected intravenously were much less effective in transmitting the disease than spleen and lymph node. Tumor tissue and splenic tissue subcutaneously injected were about equally effective in producing subcutaneous tumor nodules. Exposure of mice to 400 roentgens preceding the injection resulted in a greater percentage of successful subcutaneous inoculations. Unrelated mice of two different stocks were resistant to transmission of the disease. Exposure of these mice to 400 roentgens has not rendered them susceptible to the disease. Mice have been negative following intravenous reinjection of leukemic splenic material. The almost complete absence of eosinophils in the leukemic infiltrations indicates that these cells are not responsible for the green color. The most intense green color is shown by the lymph nodes, while the subcutaneous tumors, which are composed almost exclusively of malignant leukemic cells, are gray with only a faint greenish hue.

FROM AUTHORS' SUMMARY.

CARCINOGENIC EFFECT OF PAPILLOMA VIRUS ON TARRED SKIN OF RABBITS. P. ROUS and J. G. KIDD, *J. Exper. Med.* **67**:399, 1938.

When the virus of the Shope papilloma is distributed by way of the blood stream to the tarred epidermis of domestic rabbits, it elicits carcinoma forthwith, as well as papilloma in great variety. The phenomenon will be analyzed, with the aid of additional instances in succeeding papers.

FROM AUTHORS' SUMMARY.

COURSE OF VIRUS-INDUCED RABBIT PAPILLOMAS AS DETERMINED BY VIRUS, CELLS AND HOST. J. G. KIDD, *J. Exper. Med.* **67**:551, 1938.

An experimental analysis of the factors responsible for the observed differences in the course of virus-induced papillomas in rabbits has shown that some of the factors are referable to the virus, others to the cells and yet others to the host. The interplay of these factors affords enlightenment of the nature of the cell-virus relationship in virus-induced tumors. Retrogression of the papillomas appears to be consequent on generalized resistance of host origin, elicited by and directed against the proliferating virus-infected cells.

FROM AUTHOR'S SUMMARY.

UTERINE ADENOMA IN THE RABBIT. H. S. N. GREENE and J. A. SAXTON JR., *J. Exper. Med.* **67**:691, 1938.

Eighty-three cases of an adenomatous tumor of the uterine mucosa have been observed in a colony of rabbits during the past four years. The results of a clinical and pathologic study of the tumor, together with a description of transplantation experiments, are included in the present report. The clinical histories of the tumor-bearing animals are similar: A long period of reproductive disturbance precedes the discovery of the tumor, and the tumor shows slow, continuous growth, with metastasis, terminating in death, in all animals held under observation for longer than one year. Microscopically, the tumor shows an atypical alveolar structure and in its characteristics closely resembles adenocarcinoma of the uterine fundus in women. Pathologic changes similar to those observed in mice following treatment with estrogenic substances are observed in the thyroid, adrenal, pituitary and mammary glands. Intraocular transplantation of the tumor has been successful, and at the present time the growth has been carried through six generations by serial transfer.

FROM AUTHORS' SUMMARY.

ECTODERMAL LESIONS PRODUCED BY THE VIRUS OF ROUS SARCOMA. E. V. KEOGH, *Brit. J. Exper. Path.* **19**:1, 1938.

The virus of Rous sarcoma has been propagated for thirty generations on the chorioallantoic membranes of developing chicks. In the chorioallantois the virus gives rise to purely ectodermal focal lesions. When dilute suspensions of the virus are inoculated, mixed mesodermal and ectodermal lesions arise. The virus may be titrated by enumerating the discrete lesions. Following inoculation of emulsions of passage membranes bearing discrete ectodermal lesions into fowls, typical Rous sarcoma appears.

FROM AUTHOR'S SUMMARY.

TUMORS IN RATS AND MICE FOLLOWING INJECTION OF THOROTRAST. F. R. SELBIE, *Brit. J. Exper. Path.* **19**:100, 1938.

Tumors can be readily induced in rats and mice by subcutaneous injection of thorium dioxide. Of rats surviving fifty-two weeks after the injection of 0.6 cc. of thorium dioxide, tumors were present in 58 per cent, and of mice surviving fifty-two weeks after the injection of 0.2 cc. of thorium dioxide, tumors were present in 26 per cent. It is suggested that the carcinogenicity of thorium dioxide is due not only to its radioactivity but also to the susceptibility of the inflammatory tissue which it produces.

FROM AUTHOR'S SUMMARY.

EFFECT OF X-RADIATION ON THE BLOOD AND LYMPHOID TISSUE OF TUMOR-BEARING ANIMALS. J. R. CLARKSON, W. V. MAYNEORD and L. D. PARSONS, *J. Path. & Bact.* **46**:221, 1938.

The growth of a mouse sarcoma was more rapid and the tumor of larger size in generally irradiated than in nonirradiated rats. Grafts into successive generations showed a higher percentage of "takes" in irradiated than in non-

irradiated rats. Investigation into the cause of the mortality of irradiated animals showed that general irradiation produces diminution in the lymphoid tissue throughout the body and that in lethal or sublethal doses it causes marked anemia, with structural changes in the lymph glands, more particularly the mesenteric, fatty changes in the liver and kidneys and atrophy of the spleen. The structural changes in the lymph nodes appear to convert these from normal lymph into hemolymph glands, concerned with phagocytosis of red cells. The deposit of iron in the tissues of irradiated animals and in those bearing primary tumors is discussed with special reference to the destruction of blood found in the lymphoid tissue of irradiated and nonirradiated mice in which sarcomas developed after treatment with a chemical compound.

FROM AUTHORS' SUMMARY.

VARIETIES OF CARCINOMA OF THE UPPER LIP. J. DELARUE AND C. FAYEIN, *Bull. Assoc. franç. p. l'étude du cancer* 27:8, 1938.

Since 1922 carcinoma of the upper lip has been seen in 32 patients at the Cancer Institute of the Faculty of Medicine, in Paris. In 18 of these the growth originated in the cutaneous part of the lip; in 3 it grew from the adjacent skin into the lip. In all the patients except 1 with a spinocellular carcinoma, which metastasized to the lymph nodes, the carcinomatous growths responded very well to roentgen therapy. In 5 instances the carcinoma developed from the mucous membrane, and in 6 it straddled the mucocutaneous junction. This type showed more marked histologic irregularities than the cutaneous type; in most of these instances the carcinoma metastasized to submaxillary lymph nodes; in a certain instance it metastasized also to the preauricular lymph node on the side of the tumor. In the cases of carcinoma of the upper lip originating from the skin the growth behaved like cancer of the face in general. It was not related to sex. Metastases were rare; local roentgen therapy was highly successful. The prognosis in such cases is good. Carcinoma of the mucosa behaved like cancer of the buccal mucosa. It was more common in man (11 cases in a series of 12). It grew and metastasized rapidly. The rarity of carcinoma of the upper lip is evidenced by the fact that in the period during which 200 cases were observed in which carcinoma originated from the mucous membrane of the lower lip only 11 cases were encountered in which it originated from the upper lip.

I. DAVIDSOHN.

LYMPHATIC RETICULOENDOTHELIOMA OF THE UTERUS. J. L. NICOD, *Bull. Assoc. franç. p. l'étude du cancer* 27:14, 1938.

In an enlarged uterus that was removed following a diagnosis of fibroma, the fundus appeared diffusely thickened, but there was no circumscribed tumor. Histologic sections revealed a network of lymph vessels lined with flat endothelial cells and filled partly or even completely with proliferating large cells, containing dark nuclei and abundant, occasionally vacuolated cytoplasm. In a few places, syncytium-like masses were present. Mitotic figures were absent. Nicod found only one similar, but not identical, case reported in the literature.

I. DAVIDSOHN.

SEMINOMA OF THE TESTIS. J. H. DEITERMANN, *Frankfurt. Ztschr. f. Path.* 50: 231, 1937.

Deitermann reviewed 27 cases of seminoma of the testis from the standpoint of histologic characteristics and clinical malignancy. He could find no support for the theory that these growths are teratomatous in origin. Their malignancy is unpredictable from the histologic appearance except that in general they are less malignant than carcinoma of the testis. Thirty-seven per cent of the patients were living and well at the end of five years.

L. OHRINGER.

A SOLITARY AMYLOID TUMOR OF THE PARIETAL BONE. U. BUERGI, Frankfurt. Ztschr. f. Path. **50**:410, 1937.

A 59 year old man had a fist-sized tumor of the parietal bone, which followed a traumatic injury of the skull and was diagnosed clinically as sarcoma. Roentgen treatment was ineffective. The tumor was removed, and the patient died. The tumor was chiefly composed of non-nucleated irregular structures, surrounded by granulation tissue containing many capillaries. These structures gave all the staining reactions for amyloid. The zones at the border of the tumor revealed that the primary deposition of amyloid occurred in the bone marrow and in the walls of the arteries of the bone marrow. No amyloid was present in any other organ. The author suggests that the deposition of amyloid was provoked by the traumatic lesion of the parietal bone. Similar tumors reported in the literature involved chiefly the vertebrae, ribs, sternum and skull. Tumors of this description are resistant to roentgen treatment and can be diagnosed only on biopsy.

ANNEMARIE STRAUSS.

NEOPLASTIC CHARACTER OF LEUKEMIA. K. APITZ, Virchows Arch. f. path. Anat. **299**:1, 1937.

In the course of one year there came to necropsy in Rössle's institute 15 persons dead of myelosis or lymphadenosis; in 7 (4 with myelosis and 3 with lymphadenosis) these conditions were not associated with tumor formation. In the remaining 8, diffuse myeloid or lymphoid hyperplasia or leukemic infiltration of the internal organs was associated with local tumor formation. The observations on these, together with the results of biopsy of tissue removed from 2 other persons, form the basis of Apitz' thesis that leukemia whether considered as myelosis or as lymphadenosis is a malignant neoplastic condition rather than a non-neoplastic hyperplasia of the myeloid or lymphoid tissues. As criteria of malignancy he stresses metastasis and aggressive growth. The 10 cases described and discussed in great detail fall into two main groups. The first comprised 3 cases of myelosis and 3 of lymphadenosis in which there developed tumor-like masses of malignant character or local aggressive proliferation. The second group consists of 4 cases of lymphosarcoma with terminal lymphadenosis of the leukemic type, held by many to be a systemic disease. The first 3 cases of the first group are held to be cases of myelosis with terminal development of myelosarcoma; the second 3 of the first group, cases of lymphadenosis terminating in lymphosarcoma. The cases of the second main group are held to be instances of lymphosarcoma with metastatic lymphadenosis. Apitz maintains that his investigations establish the neoplastic character of the myeloses and lymphadenoses, usually included under leukemia. The appearance of the proliferated cells in the peripheral blood is of clinical significance but not of essential differential importance. He proposes the following purely descriptive classification:

I. Lymphoid hemoblastoses

1. Lymphosarcoma of localized type capable of forming hematogenous or lymphogenous metastases (Ghon-Roman type)
2. Lymphosarcomatosis with regional involvement and without a recognizable primary focus (Kundrath-Paltauf type)
3. Lymphosarcoma with metastatic lymphadenosis
4. Lymphadenosis with terminal development of lymphosarcoma
5. Simple lymphadenosis, in which the aggressive character of the growth is evident only microscopically
6. Combinations of lymphosarcoma and lymphadenosis in which it is impossible to determine which was the primary condition.

II. Myeloid hemoblastoses

1. Myelosis with later development of myelosarcoma
2. Myelosis with localized aggressive growth or metastatic nodules (chloroleukemia)
3. Simple myelosis, in which aggressive growth is detectable only microscopically.

O. T. SCHULTZ.

THE IMMUNOLOGIC RELATIONSHIP BETWEEN THE SHOPE FIBROMA VIRUS, THE VIRUS OF MYXOMA AND NEUROLAPINE VIRUS. KO-DA GUO, Zentralbl. f. Bakt. (Abt. 1) **139**:308, 1937.

Experiments showed a definite relation between the neurovirus obtained from rabbits (neurolapine) and fibroma virus but none between the former and the virus of myxoma. Rabbits which had withstood a cutaneous invasion by the neurovirus obtained from rabbits exhibited local immunity to infection in the same area with the fibromatosis strain of the Shope fibroma virus, but when the latter virus was injected into other areas of skin infection developed. Rabbits which had withstood a cutaneous infection with neurolapine virus were also immune to infection with the inflammatory strain of the Shope fibroma virus but not to a later infection with the virus of infectious myxomatosis. A previous infection with Shope fibroma virus engendered no resistance to infection with myxoma virus.

PAUL R. CANNON.

Society Transactions

CHICAGO PATHOLOGICAL SOCIETY

KATHARINE M. HOWELL, *President*

Regular Monthly Meeting, Nov. 14, 1938

EDWIN F. HIRSCII, *Secretary*

PREPARATION OF M AND N TESTING FLUIDS. I. DAVIDSOHN.

In this paper I present my experience in the preparation of anti-M and anti-N immune serums and testing fluids. The details of the technic found useful are given. Boiled blood of type M (OM, A₁M and BM) was found well suited for the preparation of anti-N testing fluids. It can replace entirely raw blood of type M. On the other hand, for the preparation of anti-M testing fluid raw blood of type N must be used, because the latter does not resist boiling.

Anti-M and anti-N immune serums and testing fluids were kept frozen without preservatives for over six months without deterioration. Their value in the determination of the blood types M, N and MN for exclusion of paternity was discussed.

The report will be published in full in the *American Journal of Clinical Pathology*.

CONGENITAL ABSENCE OF THE PENIS. GEORGE J. RUKSTINAT and ROBERT HASTERLIK.

A male baby, 41 cm. long and weighing 2,020 Gm., born prematurely with a footling-breech presentation, had the following abnormalities: absence of the anus, penis and prostate gland and of the median raphe of the scrotum; a persistent atretic communication between the base of the bladder and the blind end of the distal part of the colon, which was hugely dilated; extensive fibrous and cystic changes of the kidneys; stenosis of both ureters; clubfoot on the left; displacement of the ossification centers of the transverse processes of the fifth lumbar and upper three sacral vertebrae. The right kidney measured only 8 by 10 by 10 mm.; the left, 15 by 12 by 10 mm. Both were nodular with cysts, which comprised about a third of the parenchyma. The remainder of each kidney consisted of heavy fibrous connective tissue strands which ensnared groups of glomeruli and collecting tubules. Most of the glomeruli were markedly altered and had thickened Bowman capsules and shrunken capillary tufts. The dilated renal tubules contained numerous lymphocytes mixed with debris and green globular material resembling the bile in meconium. The ureters were narrowed, the right throughout most of its length and the left near the ureteropelvic junction for 3 mm. The wall of the urinary bladder was up to 4 mm. thick. The lining was trabeculated and had four diverticula, about 3 mm. deep, near the fundus. On the right side, the bladder bulged sharply at a point 2 mm. superior and anterior to the ureteral opening and communicated by a passage, 0.5 mm. in diameter, with the distal part of the colon. The opening was occluded by the wrinkled mucosa of the bladder but could be forced with gentle pressure. The adherent colon was 5.5 cm. in circumference and had a wall 0.5 mm. thick. The rugae of the lining occurred at wide intervals; the mucosa was stained with bile. The ascending part of the colon, by contrast, was only 14 mm. in circumference and 1 mm. thick. The distal end of the colon was smoothly rounded and had no distal outlet except at the narrow passage into the bladder. There was no vestige of a penis either externally or internally. The left testicle was in the scrotum, and the right was at the middle of the right psoas muscle.

DISCUSSION

P. GRUENWALD: Cases of atresia of the anus and absence of the penis are of great importance since Feller and Sternberg published their paper on sirenid malformations, in 1931. Such malformations are defined as developing from median and symmetric defects of the anlage of the caudal end of the body. The group described was named after the best known subgroup, the sirens, which are characterized by a fusion of the legs. Feller and Sternberg have already stated, in their first report, that those defects can give origin not only to malformations characterized by fusion of the legs but also to many other anomalies of the pelvic region, e. g., atresia of the anus or absence of the penis. I think that the knowledge of the different types of sirenid malformations will be helpful in the clarification of many of the various malformations of the pelvic region. Therefore the case presented has great importance.

O. SAPHIR: Were there any changes in the testes and what was the condition of the seminal vesicles and prostate?

S. LEVINSON: Is there a report of congenital absence of the penis without other malformations?

G. RUKSTINAT: Hemorrhages were the only changes noted in the testes. The prostate and seminal vesicles were absent. One report described this as the only anomaly in a boy. The ureters opened into the bowel. There are only twelve other reports of this malformation.

INTRACYSTIC PAPILLOMA OF THE BREAST. OTTO SAPHIR.

While minute intracystic papillomas of the breast often are present in instances of so-called chronic cystic mastitis, they also occur as independent tumors in breasts which have no other changes. Three varieties of intracystic papilloma may be differentiated: the fibrous type, the glandular type and a papilloma which consists of an insignificant connective tissue stalk covered by transitional epithelial cells resembling the mucosal lining cells of the urinary bladder (transitional cell type). A combination of these three types is seen frequently. The fibrous type is formed by a new growth of connective tissue which extends into the duct or cyst and is covered by duct lining cells. Such a tumor may be uniradicular or multiradicular. In multiradicular papilloma the epithelial cells of two adjacent rami may fuse, thus forming glandular structures. Possibly the glandular type is formed also by extension of neighboring periductile acini into a duct or cyst, the acini still being surrounded by the epithelial lining cells of the duct. Intracystic papilloma of the breast as a rule is multiple. The multiplicity may be explained on the basis of multiplicity of origin or by implantations of tumor cells in neighboring ducts. Such implants do not indicate necessarily that the tumor is malignant; the latter may be compared to certain benign ovarian tumors which occasionally produce implantations on the peritoneal surface. The multiplicity of intracystic papilloma of the breast and the consideration of the possible mode of origin of the secondary tumors should influence the choice of surgical procedure.

DISCUSSION

I. DAVIDSOHN: Intracystic papilloma and chronic cystic mastitis have been associated with disturbances of the glands of internal secretion, i. e., with the presence or absence of hormones. Two tissues, the stroma and the epithelium, are concerned, each specifically. Is hyperplasia of the epithelium present in young and absent in older patients, and were tall acidophilic acinar cells noted in the breast tissues in pregnant and not in nonpregnant women? Preparations of gonadotropic substances now being used cause changes of the mammary gland which are not spontaneous but are due to therapy.

O. SAPHIR: Papillomas are an integral part of chronic cystic mastitis. The ages of the patients whom I studied ranged between 20 and 50 years. I did not observe acidophilic cells.

MALACOPLAKIA OF THE URINARY BLADDER. ALEX B. RAGINS and DORRIN F. RUDNICK.

Malacoplakia of the urinary bladder is reported in a woman, aged 53 years. The lesions were associated with chronic ascending pyelonephritis, nephrolithiasis and chronic ureteritis. The plaques contained large polygonal cells with Michaelis-Gutmann bodies and also large accumulations of lymphocytes and plasma cells. They were entirely distinct from the transitional epithelium covering them, as shown by various differential stains.

Bacteriologic studies revealed the chief organism to be *Bacillus mucosus capsulatus* of Friedländer, which was isolated from the urinary bladder and the left ureter. On removal of the left kidney and ureter, all of the symptoms and the malacoplakia disappeared.

KATHARINE M. HOWELL, *President*

Regular Monthly Meeting, Dec. 12, 1938

EDWIN F. HIRSCH, *Secretary*

INGUINAL GRANULOMA WITH VISCERAL AND OSSEOUS LESIONS. ELEANOR M. HUMPHREYS.

The clinical and autopsy observations in a case of inguinal granuloma seem to contradict the widely held view that this disease is invariably superficial and local, accompanied by only mild general reactions. Search of the literature and a review of the few autopsy reports reveal only scanty evidence for the occurrence of generalized infection due to dissemination of the agent responsible for inguinal granuloma. In the case reported now, however, the highly characteristic Donovan organisms were demonstrated in the typical superficial lesions of the external genitalia and vicinity and in numerous lesions in the abdominal viscera, abdominal lymph nodes and bones of the thorax. The sites of these granulomatous and focally suppurative lesions were such as to indicate that they were caused by direct and deep extensions from the superficial lesions and by spread of the infectious agent through lymph and blood channels. There is no evidence that secondary bacterial infection played more than a minor role in the disease. Whether or not the pathologic observations in this case represent a rarity or exemplify merely the outcome in cases of advanced untreated inguinal granuloma will be determined only by more and careful postmortem studies.

DISCUSSION

E. A. PRIBRAM: What was the condition of the blood?

J. D. KIRSHBAUM: Was there amyloidosis?

ELEANOR M. HUMPHREYS: There was no amyloidosis. The erythrocytes of the blood were 4,120,000 and the leukocytes 14,000 per cubic millimeter just before the use of sulfanilamide. A differential examination was not made. After the use of sulfanilamide the erythrocyte count dropped to 1,200,000 and 2,000,000, and the leukocyte count, to 5,700.

HYPERNEPHIROMA OF THE OVARY. F. W. VAN KIRK JR. and E. A. EDWARDS.

In this paper a discussion of opinions concerning the origin and classification of tumors of the ovary designated hypernephroma introduces the description of a left ovarian ovoid mass, 23.5 by 20 by 12 cm., which weighed 6 pounds and 2 ounces (2,778 Gm.). The patient, aged 42 years, had as her chief complaints

frequency of menstruation, constipation and a growing tumor mass in the abdomen. There were no changes of the secondary sex characteristics. Nineteen months after the operation there was no recurrence. All clinical observations indicated that the tumor was primary in the left ovary. On surfaces made by cutting the tumor there were a few cystlike pockets, ranging from 3 to 6 cm. in diameter, in a solid tissue composed of gray and yellow portions, the latter necrotic. The tumor tissues had large pale granular cells with sharp cell borders, arranged in alveoli and was like renal hypernephroma. The cytoplasm contained abundant glycogen. The glycogen content of the moist tissues on fixation in solution of formaldehyde U.S.P. was by chemical analysis 1.78 per cent.

DISCUSSION

WALTER SCHILLER: Among the hypernephroid tumors is the true hypernephroma, which originates either from the cortex of the adrenal gland in the normal location or from an island of adrenal cortex misplaced by developmental error into the cortex of the kidney. Such a neoplasm is real adrenal hypernephroma. There is another type of tumor, originating from the tubular region of the kidney and consisting of large polyhedral cells with a protoplasm full of fat droplets, which sometimes almost duplicates the true hypernephroma in appearance but otherwise has a marked tendency to form adenomatous or cystic structures and papillary projections. This type, since it originates from the renal cortex and secondarily duplicates only the structure of the hypernephroma, as Stoerk described, should not be called hypernephroma but simply hypernephroid. In the ovary both types of tumors can be found—the hypernephroma and the hypernephroid tumor. The case of Ruston, because of the marked papillary structure of the tumor, should be classified as an instance of hypernephroid.

The differentiation between the hypernephroma and the luteinized granulosa cell tumor can be made not only by comparison of the tumor cells but by analysis of the surrounding connective tissue. In the granulosa cell tumor the surrounding connective tissue has close functional and morphologic relations to the tumor tissue, duplicating the relation between the physiologic theca and the physiologic granulosa. In the adrenal cortex the framing connective tissue forms a neutral capsule only, which is not reactive to the activities of the included cortical tissue. The same holds for the hypernephroma. The tumor demonstrated by Dr. Van Kirk shows the absence of any but mechanical relations between the connective tissue capsule and the tumor tissue.

O. SAPHIR: The origin of some of the cells in the ovary is not exactly known and hence the origin of a tumor which arises in this tissue is not certain. I believe the differentiation between the true hypernephroma and the luteoma should be on the basis of biologic assay.

SYSTEMIC MYCOSIS WITH MYCOTIC ENDOCARDITIS. NATHAN B. FRIEDMAN and LILIAN DONALDSON.

A 57 year old Illinois grocer suffered from malaise and afternoon fever for a year before his death. Physical examination led to a diagnosis of subacute bacterial endocarditis, but the blood cultures were negative. The autopsy showed a luxuriant vegetation on the aortic valve, which microscopically proved to be filled with nests of yeastlike cells, and scattered through many organs were miliary tuberculoid granulomas.

DISCUSSION

E. A. PRIBRAM: One does not know how to classify this organism, because it was not identified culturally. Is there any possibility that the disease was tularemia?

O. SAPHIR: Perhaps the yeastlike organism is a secondary invader, engrafted on some other infection.

PRIMARY ADENOCARCINOMA OF THE JEJUNUM WITH PERFORATION. MAURICE B. JACOBS and E. A. CHRISTOFFERSON.

Primary carcinoma of the jejunum is rare. An acute perforation is still more unusual. Only one case has been reported heretofore in the last eight years. The present instance concerns a white man, aged 48 who was admitted to the Cook County Hospital on May 1, 1935, complaining of sudden severe abdominal pains. There had been no previous complaints referable to the gastrointestinal tract. His temperature was 100.6 F. rectally; the pulse rate, 102; the respirations, 36 per minute, and the blood pressure, 132 systolic and 80 diastolic. The abdomen was hard, slightly distended and diffusely tender on palpation. Peristaltic sounds were absent except for an occasional metallic click. Fluoroscopic examination revealed free air beneath the right dome of the diaphragm. A preoperative diagnosis of perforated peptic ulcer was made. Twenty-six hours after the onset of pain, a perforation of the jejunum 18 inches (45.7 cm.) distal to the ligament of Treitz was found. The regional lymph nodes were firm and enlarged. A diagnosis of carcinoma was made, and wide resection of the involved tissues together with the mesentery was done. Histologic examination revealed the tumor mass in the jejunum to be adenocarcinoma. Four days after the operation the patient died, and the postmortem examination demonstrated generalized fibrinous peritonitis and metastases in the mesenteric and periaortic lymph nodes. Both lower lobes of the lungs showed bronchopneumonia.

The literature was reviewed, and only one other case, observed by Dencks, was found recorded. In 10,309 consecutive necropsies at the Cook County Hospital between 1929 and 1938 only 7 cases of carcinoma of the small intestine were encountered, of which 3 were in the duodenum and 4 in the jejunum. Combined statistics from hospitals of Vienna, Baltimore and Chicago, covering 90,937 necropsies, included only 58 cases of primary carcinoma of the small intestine and in only 8 of these was the growth in the jejunum.

DISCUSSION

ALEX B. RAGINS: Up to 1938, 3 cases of malignant tumor of the small bowel in addition to the 7 cases described by Drs. Jacobs and Christofferson were observed at the Cook County Hospital. Two of these were instances of reticulum cell lymphosarcoma. In the first the tumor was located in the second portion of the duodenum, involving the ampulla of Vater, and in the second the tumor involved portions of the entire small bowel, starting from the jejunum, extending through the ileum and ending abruptly at the ileocecal junction. The third tumor was a pedunculated leiomyosarcoma of the ileum. Since observing these, I have encountered 3 more instances of sarcoma of the small bowel in which the growth had been resected surgically. In one the tumor was in the jejunum and in the others it was in the ileum. In the former the growth was lymphosarcoma. In the latter 2 it was reticulum cell lymphosarcoma. My associates and I have found carcinoma occurring more frequently in the small intestine, where most investigators have shown a marked prevalence of lymphosarcoma over carcinoma.

Book Reviews

The Special Pathological Anatomy and Pathogenesis of the Circulatory, Renal and Digestive Systems Including the Liver, Pancreas and Peritoneum. Horst Oertel, Strathcona Professor of Pathology, Director of the Pathological Institute, McGill University, and Pathologist to the Royal Victoria Hospital, Montreal, Canada. Pp. 630. Price \$8.50. Montreal, Canada: Renouf Publishing Company.

This book is the outgrowth of a series of notes for the author's lectures, which have been expanded, elaborated and clarified in Professor Oertel's usual scholarly style. The preface and introduction are philosophic essays, based on wide reading and experience in educational problems, well worthy of perpetuation in this or any other form. The text is written with historical perspective. Emphasis is laid on etiology and pathogenesis, and there is furnished a thorough survey of pathologic morphology and function. For Oertel, pathology is a science of and for itself, but even so he is familiar with its practical bearing, and the facts and discussions are so presented that the book has genuine value for both the clinician and the pathologist. It should also be useful to those among the medical students whose interest is in the subject rather than merely in the final examination. The book reads smoothly, and the matter is fluently put, but it must be said that the writing is by the large, as Oertel says, "discursive." This formula gives him ample opportunity for full critical and informative discussion. Clarity, thoroughness and balance in the treatment of the subject matter prevail throughout.

Special or systemic pathology is of the deepest interest to those who would use this body of knowledge in the practice of medicine. Special pathology, however, in order to be sound, must be built on a solid foundation of general pathology, and most important in this broad field is knowledge of inflammation, of its nature and processes. Oertel is an adherent of Ricker's hypothesis of the nervous control of inflammation, and naturally this permeates his discussions of numerous special topics. Ricker and Oertel may be right, but many pathologists here and abroad do not think so. A book review is no place to argue the question, but the subject assumes indubitable importance in the mind of any teacher of pathology who advises his students in the selection of a textbook. Much the same criticism may be directed toward highly individualistic descriptions and classifications, such as those of Bright's disease.

With due consideration, and probably with the idea that specimens should be readily available to the student, Oertel has provided no pictorial illustrations. To be sure, a high grade laboratory should have plenty of illustrative material, but in not a few institutions the small volume of pathologic material and the cost of preservation of the material in suitable form are handicaps too great for the collection of those typical lesions which are so essential to the student's fund of information. Experience has shown that in nearly all fields of study the word picture, admirable as it may be, often requires amplification by illustrations. The value of a textbook on almost any subject is enhanced by well selected photographs, drawings and the like.

Although Oertel makes no claim for completeness, the references listed at the end of each chapter are well selected and on the whole reasonably extensive. Oertel is devoted to Germanic science and philosophy, a quality which is reflected in the numerous excellent summaries of what these peoples have contributed to pathology and medicine. Indeed, there is a suggestion of overemphasis on the work of Continental investigators in relation to the newer American school. The reviewer protests that in making this criticism he is not guided by undue nationalism but by a desire to see that all good things are included.

The format of the book is somewhat larger than is usual in this type of publication; the paper is of good mat surface, the type is large and the printing clear and uniform. The book is interesting and enlightening reading for those advanced students of pathology and medicine who are sufficiently experienced to formulate their own judgments of the expositions of a highly respected author.

Pathological Technique. A Practical Manual for Workers in Pathological Histology Including Directions for the Performance of Autopsies and for Microphotography. Frank Burr Mallory, A.M., M.D., S.D., Consulting Pathologist to the Boston City Hospital, Boston, Mass. Cloth. Pp. 434, with 14 illustrations. Price \$4.50. Philadelphia: W. B. Saunders Company, 1938.

Receiving this book was just like meeting again an old friend whom one missed badly and for whose return one waited impatiently. The pleasure of the meeting was enhanced by evidences of rejuvenation and up-to-dateness, much more fundamental than a mere face lifting.

The reviewer's affection in approaching this book will be understood and, he hopes, excused by all who received their training in pathology during the past generation, when the names Mallory and Wright were synonymous not with a book on pathologic technic but with the subject of pathologic technic. When the book, only twenty-seven years old, reached in 1924, with its eighth edition, the enviable position of a standard in its field, the authors or the publishers did not respond to a general demand for a new edition, and soon the book could not be obtained. Other books were published, but none of them was able to replace the old standby. But here it is, dedicated to the memory of the former co-author, James Homer Wright.

It is a new book, though retaining all that was good in the old one. It has been brought up to date and considers all that has taken place since 1924, all that has proved valuable or that shows the promise to stay.

The book has gained by the changes to which it has been subjected; it has become truly a book on pathologic technic through the elimination of large chapters devoted to bacteriology, serology and hematology.

The subject matter is treated in three parts and nineteen chapters. The first part, 105 pages, deals with general histologic methods, including the examination of unfixed material and the processes of fixation, decalcification and embedding of tissues. The relatively new dioxane method is presented; then follow discussions of the stains, natural, artificial and metallic, of the clearing and mounting reagents, of microincineration and of injection methods. The special histologic methods as they are used for the study of the cell and of its component parts, of the different special cells and tissues and of the various organs are taken up in three chapters of the second part. The nervous system is treated exhaustively in 45 pages. Two chapters are given to bacterial stains and to infectious agents, such as actinomycetes, yeasts and molds, rickettsias, filtrable viruses, spirochetes, protozoa and worms. The sixteenth chapter of part three takes up the technic of the necropsy in 73 pages. The different methods are presented and their relative advantages discussed. This chapter contains a wealth of material in a limited space. Then follow brief and valuable chapters on the preservation and mounting of gross specimens, on gross and photomicrography and on the making of lantern slides, and finally with suggestions for such practical matters as the blackening of table tops and the cleaning of glassware. A well selected 8 page bibliography and a 28 page index conclude the book. The type and binding are satisfactory.

There is every reason to expect that this book will prove a worthy successor to the old "Mallory and Wright." No pathologist, no laboratory technician can afford to be without it. It is hoped that the author will see the editions multiply as he did in the case of the older book.

Books Received

NATIONAL RESEARCH FELLOWSHIPS 1919-1938. Physical Sciences, Geology and Geography, Medical Sciences, Biological Sciences. Pp. 95. Washington, D. C.: National Research Council, 1938.

LE DÉSÉQUILIBRE ALIMENTAIRE (CARENCE C.) DANS LES TROUBLES DU MÉTABOLISME CALCAIRE (OSSIFICATION ET DENTITION) EN PATHOLOGIE HUMAINE ET COMPARÉE. Georges Beltrami, ancien interne des hôpitaux, professeur à la Faculté de Médecine de Marseille. Pp. 35. Marseille: M. Leconte, 1938.

HUMAN PATHOLOGY. A TEXTBOOK. Howard T. Karsner, M.D., Professor of Pathology, Western Reserve University, Cleveland. With an introduction by Simon Flexner, M.D. Fifth edition, revised. Cloth. Pp. 1013, with 461 illustrations. Price, \$10. Philadelphia and London: J. B. Lippincott Company, 1938.

MANUAL OF VETERINARY BACTERIOLOGY. Raymond A. Kelser, D.V.M., A.M., Ph.D. Third edition, thoroughly revised. Cloth. Pp. 640, with 93 illustrations. Price, \$6. Baltimore: Williams & Wilkins Company, 1938.

CANCER WITH SPECIAL REFERENCE TO CANCER OF THE BREAST. R. J. Behan, M.D., Dr.Med. (Berlin), F.A.C.S., Co-Founder and Formerly Director of the Cancer Department of the Pittsburgh Skin and Cancer Foundation, Pittsburgh. Cloth. Pp. 844, with 168 illustrations. Price, \$10. St. Louis: C. V. Mosby Company, 1938.

CANCER. ITS DIAGNOSIS AND TREATMENT. Max Cutler, M.D., Associate in Surgery, Northwestern University Medical School; Chairman, Scientific Committee, Chicago Tumor Institute; Consultant, Tumor Clinic, and Director, Cancer Research, U. S. Veterans Administration, Hines, Ill., and Franz Buschke, M. D., Assistant Roentgenologist, Chicago Tumor Institute; Late Assistant, Roentgen Institute, University of Zurich. Assisted by Simeon T. Cantril, M.D., Director, Tumor Institute, Swedish Hospital, Seattle; Late Assistant, Chicago Tumor Institute. Cloth. Pp. 757, with 346 illustrations. Price, \$10. Philadelphia and London: W. B. Saunders Company, 1938.

ARCHIVES OF PATHOLOGY

VOLUME 27

MARCH 1939

NUMBER 3

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MECHANISM OF LOCALIZATION OF VEGETATIONS OF BACTERIAL ENDOCARDITIS

ARTHUR C. ALLEN, M.D.

NEW YORK

In studying vegetations in cases of acute and of subacute bacterial endocarditis¹ one is impressed by the constancy with which they are located on the valve surface facing the chamber which the valve serves, i. e., the outflow surface. The vast majority show predilection for the line of closure, but not infrequently the vegetations cover the greater part of the outflow surface. This has been appreciated for many years (Osler,² Clawson,³ Clawson and others⁴). It seems, therefore, that nature must engineer such constancy by a definite mechanism rather than by chance distribution. However, from the diversity of the theories one may conclude that the principles of this mechanism have not been settled. The concepts that have been suggested may be outlined as follows: (1) coronary embolism, (2) mechanical forces such as eddy currents or impingement of valvular margins and (3) miscellaneous theories.

THEORIES

Coronary Embolism.—As far back as 1863 Luschka⁵ described blood vessels in valves and correlated his findings of a richer vascularity in the mitral and aortic valves with the well established fact that endocarditis is most commonly found in these structures. This view was opposed at that time with the very modern objection that such valves were the seat of disease and thereby achieved their vascularity. However, in 1909 Coombs⁶ presented reasons for believing that the organ-

From the Department of Pathology, Cook County Hospital; director, Dr. R. H. Jaffé (deceased).

1. Allen, A. C.: Nature of the Vegetations of Bacterial Endocarditis, Arch. Path., to be published.

2. Osler, W.: Lancet **1**:415, 1885.

3. Clawson, B. J.: Arch. Int. Med. **33**:157, 1924.

4. Clawson, B. J.; Bell, E. T., and Hartzell, T. B.: Am. J. Path. **2**:193, 1926.

5. Luschka, cited by Kerr and Mettier.⁸

6. Coombs, C.: Lancet **1**:1377, 1909.

isms reach the valve through the coronary vessels. In 1917 Bayne-Jones⁷ advanced evidence to show that valves are normally vascularized. This was confirmed by Kerr and Mettier⁸ who injected india ink into the coronary circulation. They concluded that their studies lent "further confirmation to the embolic concept of endocarditis."

Recently Wearn and Moritz⁹ published pertinent data from detailed studies of the vessels of valves. Of 235 hearts with no apparent inflammation, the mitral valve was vascularized in 50 per cent, the tricuspid valve in 31 per cent, the aortic valve in 5 per cent and the pulmonary valve in 4 per cent.

According to the embolic concept of endocarditis, one logically expects those valves to be most frequently involved which are most frequently vascularized. However, among apparently normal valves, the aortic was found vascularized far less often than any of the others and with about one-sixth the frequency of the tricuspid. And yet the tricuspid valve is the seat of endocarditis far less often than the aortic, even without antecedent rheumatic disease.

An additional objection—among others—to the coronary theory lies in the totally inadequate explanation for the localization of lesions on valves rather than on mural endocardium, not to mention the predilection for valves on the left side of the heart, and the constant selection of a particular part of the valve. The last part of this objection Eisenmann¹⁰ meets with the statement that such highly selective localization depends on a peculiar distribution of the coronary vessels. Not only has this not been confirmed by subsequent elaborate studies but, as a matter of fact, there seems currently to be some doubt (Gross¹¹) that normal valves are vascularized at all. It may then be concluded that this theory lacks adequate evidence as an explanation for the great majority of cases.

Eddy Currents.—Inasmuch as many of the lesions appear to begin near the line of closure, the explanation for the localization has been sought in eddy currents. But do eddy currents occur at this point? It is a fundamental physical principle that eddy currents tend to occur in a region of diminished pressure, particularly within a pocket. Adami and Nicholls¹² long ago pointed out that thrombi generally originate in venous valve pockets because of these currents. In the heart, pockets occur in two principal places (fig. 1): (1) between the superior

7. Bayne-Jones, S.: *Am. J. Anat.* **21**:449, 1917.

8. Kerr, W. J., and Mettier, S. R.: *Am. Heart J.* **1**:96, 1925.

9. Wearn, J. T., and Moritz, A. R.: *Am. Heart J.* **13**:11, 1937.

10. Eisenmann, cited by Hutyra, F., and Marek, J. F.: *Special Pathology and Therapeutics of the Diseases of Domestic Animals*, Chicago, Alexander Eger, 1912, vol. 1, p. 1080.

11. Gross, L.: *Am. Heart J.* **13**:275, 1937.

12. Adami, J. G., and Nicholls, A. G.: *The Principles of Pathology*, Philadelphia, Lea & Febiger, 1909, vol. 2, p. 68.

surface (or fibrosa) of the semilunar valves and the great vessels and (2) between the ventricular surfaces (or fibrosa) of the auriculoventricular valves and the wall of the ventricle. These are the sites of eddy currents (Best and Taylor¹³). However, both these areas are only exceptionally the seat of primary vegetations. It must therefore be concluded that eddy currents play no apparent significant role in the initial localization of bacterial vegetations.

Impingement.—According to this theory, the line of closure is frequently elected because of the trauma and strain due to mechanical impingement of the margins against each other as the leaflets or cusps slap shut (Leschke¹⁴; MacCallum¹⁵; Nedzel¹⁶). However, does significant impingement occur? To answer this question, it is important to review the anatomic background of bacterial endocarditis.

It is generally agreed (Libman¹⁷; Clawson³; Thayer¹⁸; Blumer¹⁹ and others) that bacterial endocarditis occurs on a previously existing

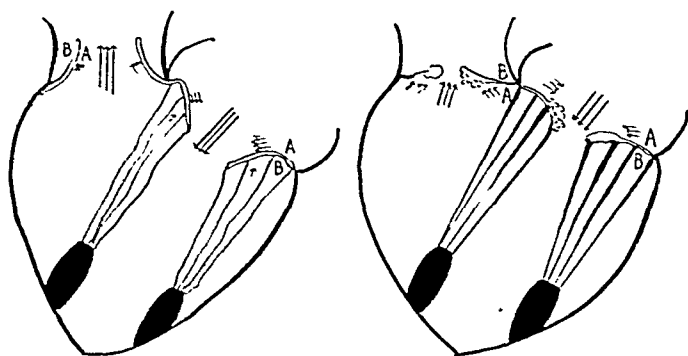


Fig. 1.—Diagrams illustrating the fact that very much more blood comes in contact with the outflow (A) than the the opposite (B) surface. It is on the outflow surface that vegetations localize with remarkable constancy. (Diagrams modified after Wiggers.)

valvular deformity in from 50 to 75 per cent of the cases. Such a deformity is usually a rheumatic fibroplastic distortion and occasionally a congenital anomaly. As is well known, the rheumatic deformity almost pathognomonically involves the free margins of the valves

13. Best, C. H., and Taylor, W. B.: *Physiological Basis of Medical Practice*, Baltimore, William Wood & Company, 1937, pp. 275-281, 183-187 and 454.

14. Leschke, E., in Kraus, F., and Brugsch, J.: *Specielle Pathologie und Therapie*, Berlin, Urban & Schwarzenberg, 1919, vol. 2, p. 1072.

15. MacCallum, W. G.: *A Text-Book of Pathology*, ed. 6, Philadelphia, W. B. Saunders Company, 1937, pp. 240-245.

16. Nedzel, A. J.: *Arch. Path.* **24**:143, 1937.

17. Libman, E.: *J. A. M. A.* **80**:813, 1923; *M. Clin. North America* **2**:117, 1918.

18. Thayer, W. S.: *Johns Hopkins Hosp. Rep.* **22**:1, 1926.

19. Blumer, G.: *Medicine* **2**:105, 1923.

(Antischkow²⁰). A fibroplastic deformity of the free margin often produces stenosis and usually concomitant insufficiency. It is difficult to conceive, therefore, of much, if any, impingement occurring on the margins of a stenotic, insufficient valve during closure when these margins are unable even to meet (fig. 2).

This leaves at least 25 per cent of the cases of bacterial endocarditis to occur on apparently normal valves. But do even these normal valve leaflets impinge with the trauma and strain that is generally believed? The following is a description by Best and Taylor¹³ of the mechanism of closure of the auriculoventricular valve:

During auricular systole, the leaflets do not lie back against the ventricular wall but occupy a mid-position as a result of two opposing currents (the eddy currents reflected from the ventricular wall and the auricular stream). When,

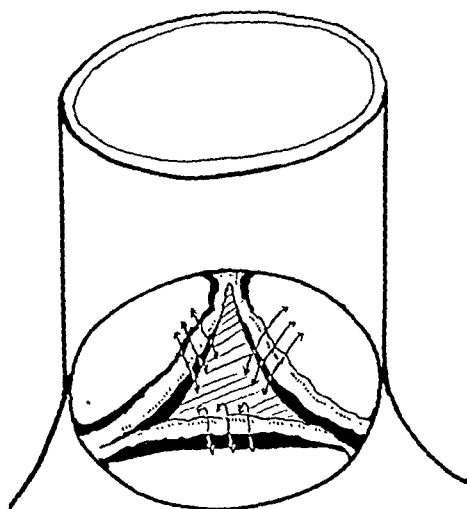


Fig. 2.—Diagram illustrating a stenotic, insufficient semilunar valve and the necessity for the line of closure to bear the brunt of increased impact since it is rigid and does not give way with the stream. Selectively increased contact and frictional resistance are also encountered in this area by virtue of the stenosis and insufficiency.

as a result of the fall in intra-auricular pressure, the incoming jet is diminished in force and finally ceases, the back eddy, persisting for a brief space and unopposed, *approximates* the valves or brings them together *gently*. They are not however firmly closed. This is effected by the rise in pressure in the ventricle when it contracts.

The trauma from closure after apposition has taken place is far less than that produced by edges which have been slapped together in the

20. Anitschkow, M.: Atherosclerosis (Lipoidosis) of the Heart Valve and Its Relation to Endocarditis, in Contributions to the Medical Sciences in Honor of Dr. Emanuel Libman, New York, International Press, 1932, vol. 1, p. 87.

manner of the general conception. Therefore, the impingement theory seems inapplicable not only to the lesions on the normal valve most frequently affected (mitral) but to the great majority of lesions superimposed on diseased valve leaflets, the margins of which are physically unable to impinge on each other.

Miscellaneous Theories.—Recently Nedzel¹⁰ reported that he was able to produce endocardial lesions by the injection of pitressin with, and rarely without, a subsequent injection of bacteria. It is his belief that the pressor episode thus brought about causes the valve to exude from its surface a stringy adhesive substance to which bacteria adhere. The trauma produced by an augmented mechanical impingement is stated to be one of the chief factors in the production of the sticky streamers, inasmuch as during the "pressor episodes, the margins of the valve impinge on each other more forcibly" (Nedzel¹⁶). And yet, it is interesting to note that all of the lesions reported were on mitral valves, where the occurrence of significant trauma during closure is open to much question, as was indicated in the foregoing paragraph. Moreover, as mentioned, in stenotic, insufficient valves, such impingement cannot occur. Nevertheless, the whole problem of pressor episodes is intriguing, and the experimental data are valuable, although one may disagree with the interpretation thereof.

Grant, Wood and Jones²¹ noted that degenerative changes tend to occur in congenitally bicuspid valves and in those with rheumatic deformities. These changes, they believe, lead to the occurrence of "platelet thrombi" which offer a foothold for bacterial implantation. This is a well recognized, keen observation. The question of whether these are truly platelet thrombi deposited on the valve or degenerative changes arising in toto from the valvular substance is not pertinent to the issue of present concern. The assumption that such projections may aid in localizing the bacteria is not unreasonable, although it does not account for the 25 or more per cent of cases of bacterial endocarditis in which the vegetations are generally assumed to occur on previously normal valves. Nor is it unreasonable to believe that the production of these changes is intimately related to the selectively greater "wear and tear" suffered by the valvular outflow surfaces where these "thrombi" were found. This will be discussed further in later paragraphs.

A variety of other possibilities have been suggested. For example, according to Babcock,²² the rheumatic valve is less vascular, with the consequence that fewer phagocytes are able to reach and destroy the bacteria. Coombs,⁶ on the other hand, believed these valves to be more

21. Grant, R. T.; Wood, J. E., and Jones, T. D.: *Heart* **14**:247, 1927.

22. Babcock, R. H.: *J. Michigan M. Soc.* **12**:645, 1913.

vascular with a resultant increase in opportunity for infection. Poynton²³ stated that the organisms lie latent in the rheumatic valve until caused to flare up with the consequent production of the characteristic bacterial endocarditis. Finally, the conception of allergy in the broadest sense of altered reactivity of tissue is gaining favor on the basis of convincing experimental evidence (Semsroth and Koch²⁴). Except the last, many such theories encounter distinct and obvious objections. However, even if these objections were met, none of the conceptions explains why the vegetations localize on one side of the deformity rather than on the other. In other words, the fibroplastic deformity, for example, in almost all instances is accessible on both sides at the distal, free margin. Similarly, the considerations of altered reaction of tissue, phagocytes and other such general factors apply more or less equally to the entire deformity. And yet, the surface facing the chamber served by the valve suffers implantation of bacteria with reliable constancy. Apparently, other factors are concerned.

In view of the multiplicity of theories, it seems desirable to set up the criterion that any theory attempting to elucidate the mechanism of localization must attempt to reconcile at least the following universally recognized facts relating to endocardial lesions:

1. In from 50 to 75 per cent of the cases, bacterial endocarditis is superimposed on a fibroplastic valvular deformity (Libman; Clawson; and others).

2. Congenital lesions are particularly susceptible to bacterial endocarditis (Abbott;²⁵ Osler²⁶).

3. There is a distinct preponderance of lesions on the left side over those on the right side.

4. Bacterial endocarditis is rare in patients with auricular fibrillation secondary to rheumatic mitral stenosis (Libman;¹⁷ Fishberg²⁷).

No single theory has been offered thus far which explains all of these facts, and several of these facts are adequately explained by none of the theories. In the remainder of the paper, an attempt will be made to show that certain mechanical principles are concerned in each of the four groups of cases just cited and, of even more importance, that these principles are the same in all.

23. Poynton, I. J.: *Brit. M. J.* **2**:306, 1920.

24. Semsroth, K., and Koch, R.: *Arch. Path.* **10**:867, 1930.

25. Abbott, M. E.: *Ann. Clin. Med.* **4**:189, 1925; *Clinical Significance of a Congenitally Bicuspid Valve*, in *Contributions to the Medical Sciences in Honor of Dr. Emanuel Libman*, New York, International Press, 1932, vol. 1, p. 1.

26. Osler, W.: *Tr. A. Am. Physicians* **1**:185, 1886.

27. Fishberg, A. M.: *Heart Failure*, Philadelphia, Lea & Febiger, 1937, pp. 338-346 and 441.

IMPACT AND CONTACT

For many years intracardiac tension has been thought to play a part in "reducing the resistance" of endocardial tissue, thus paving the way for implantation of bacteria. The occurrence of lesions in sites subjected to abnormally high tensions—for example, the right auricle with a patent foramen ovale or the right ventricle with a defective septum fibrosum—supports this general thesis. Gross,²⁸ who has treated the entire subject of endocarditis comprehensively, emphasized the factor of blood dynamics. The production of endocarditis by the injection of pitressin and bacteria (Nedzel) furnishes supporting experimental data. However, the term "hemodynamics" lends itself to facile usage. Precisely how do these dynamics bring about almost constant localization of vegetations at certain sites? The process, it is believed, entails more than simple tension. The attempt will therefore be made to analyze more exactly the nature of these dynamics, which may be resolved into two major components: (a) impact and (b) contact. Each of the four facts will now be examined separately to determine the role played by each of these components.

1. *Implantation of Vegetations on Rheumatic Endocardial Deformities.*—(a) Impact: In the normal valve, a good deal of the impact is warded off because the leaflets "give way" or play with the stream (fig. 1). In the rheumatic valve, however, the brunt of this impact is borne by the rigid, nonelastic parts, i. e., the line of closure, the usual site of the rheumatic fibroplastic deformity, because it is unable to "give" with the stream. The basis for this principle of mechanics is no less than Newton's third law of motion, which states that "to every action there is an equal and opposite reaction." In other words, the more fixed and unyielding an object is, the greater will be the impact produced against that object (Kimball²⁹).

Therefore, as a corollary to this, any condition which appreciably alters the impact must be a factor, to the degree of such alteration, in enhancing the predisposition toward the localization of bacteria. Such an increase in the impact is produced in stenosis. For example, in aortic stenosis, "the velocity of ejection is greatly increased over normal—even during rest. The work of the left ventricle may be nearly doubled and the increase is due to the enormous increase in the kinetic factor" (Best and Taylor¹³). The impact is, of course, directly proportional to the kinetic factor inasmuch as both are a function of mass X velocity (Kimball²⁹).

28. Gross, L., and Fried, B. M.: *Am. J. Path.* **13**:769, 1937.

29. Kimball, A. L.: *A College Text-Book of Physics*, ed. 4, New York, Henry Holt & Company, Inc., 1932, pp. 19-24.

In addition, insufficiency is the usual concomitant of rheumatic stenosis. According to Fishberg, the diastolic backflow in insufficiency averages 36 per cent. According to Wiggers,³⁰ this backflow may vary from 5 per cent in small leaks to 50 per cent or more. This backflow increases the diastolic volume, and this, in accordance with Starling's law of the heart, causes a greater force of contraction and hence a correspondingly greater impact. In other words, in both stenosis and insufficiency—the common complications of rheumatic valvulitis—there is brought about an increased impact against the valve. The brunt of this impact is borne by the site of special interest—the fibroplastically deformed line of closure on which bacterial vegetations are commonly implanted.

(b) Contact: If, along with increased impact, a greater number of organisms forcibly brush against a certain part of the valve, the chances for bacterial implantation on that part naturally become greater. Therefore, the question to be answered is: Which part of the valve contacts the most organisms in a bacteremia?

In the first place, many more cubic centimeters of blood come in contact with the ventricular or outflow surface (spongiosa) of the normal or the deformed semilunar valve (and the outflow or auricular surface of the auriculoventricular valve) than with the opposite surface. This "opposite surface" (fibrosa) tends to be pressed against the aortic, pulmonary or ventricular wall, depending on the valve (fig. 1), and comes in contact with only a small fraction of the area of blood met by the outflow surface. (The pocket formed by the aortic wall and the valve fibrosa [fig. 1] is much too small to make eddy currents a significant factor.) By the same token, in bacteremia many more organisms will come in contact with this ventricular surface (fig. 1). As a matter of fact, vegetations that more or less completely cover this selectively contacted surface of a cusp without extension to the opposite surface are seen not infrequently, particularly in acute lesions. According to Swift,³¹ the mitral lesions extend up over the wall of the left auricle in more than half the cases. These instances lend support to the concept that selective contact between valve and organisms occurs in this location and is important in the localization of vegetations. This same description of mechanism applies to each of the other valves.

Such is the situation when the valves are unthickened. However, a brief review of the mechanics discloses that in the stenosis and insufficiency produced by a rheumatic deformity there is brought about contact with an increased number of organisms at the line of closure.

30. Wiggers, C. J.: J. A. M. A. **97**:1359, 1931.

31. Swift, H. F.: Endocarditis, in Nelson Loose-Leaf Living Medicine, New York, Thomas Nelson & Sons, 1932, vol. 4, p. 323.

In stenosis, three factors tend to work in this direction: (1) the diminished diameter of the stream as it passes through the narrowed orifice, (2) the increased stroke volume (Wiggers³²) and (3) the lengthened systole (Wiggers³²). The first two factors obviously increase the total surface area and hence the total number of organisms that rub against the projected portion or line of closure of the valve. The third factor increases the duration of such contact. All these elements by their very nature predispose toward bacterial implantation.

Over and above this, the insufficiency produces additional contact of organisms with the line of closure. This takes place during the diastolic backflow when, as the blood returns to its chambers, it again selectively brushes by the line of closure in vastly greater quantities than by the remainder of the valve (fig. 2). This diastolic backflow, as mentioned, may be considerable (from 5 to 50 per cent of the systolic discharge).

Furthermore, it is clear that any frictional resistance tends to make the contact between the valve surface and the blood stream more intimate. Such resistance is encountered in stenotic hearts. This is so because resistance is approximately proportional to the square of the velocity and inversely to the cross section of the orifice (Best and Taylor¹³). In rheumatic stenosis, there are present both an increased velocity and a narrowed orifice, each of which increases resistance, intimacy of contact and hence the chances for implantation of bacteria. The minute "platelet thrombi" described by Grant, Wood and Jones, may be an additional source of increased resistance.

So much for the majority of cases of bacterial endocarditis.

2. Susceptibility of Congenital Lesions to Bacterial Endocarditis.—The predisposition of congenitally abnormal valves to bacterial endocarditis is well known. However, in practically all such cases, with the exception of that of the bicuspid aortic valve, there is an obvious, self-explanatory defect in mechanics whereby abnormally great tension and impact are transmitted to a region not designed therefor, for example, a patent ductus arteriosus, a patent foramen ovale, septal defects and valvular stenosis. The predisposition of the "so-called congenitally bicuspid aortic valve," on the other hand, is not quite so easily explained. This is apparently not an uncommon finding. Thayer¹⁸ reported an incidence of 7 per cent, and Lewis and Grant³³ one of 26 per cent, of bicuspid valves in their cases of subacute aortic bacterial endocarditis. Abbott²⁵ stated that of 40 hearts with bicuspid valves, 18, or 45 per cent, showed lesions of bacterial endocarditis.

32. Wiggers, C. J.: *Circulation in Health and Disease*, Philadelphia, Lea & Febiger, 1937, pp. 686-690 and 652-653.

33. Lewis, T., and Grant, R. T.: *Heart* 10:21, 1923.

Recently Gross³⁴ emphasized convincingly that these lesions, particularly those in adults, are most often on an inflammatory (rheumatic) basis—rather than congenital—with resultant fusion of cusps. This would tend, of course, toward stenosis and insufficiency. As a matter of fact, according to Abbott, stenosis and insufficiency *are* frequent concomitants of valves of this type. On this basis, the principles outlined in the foregoing discussion of the dynamics of stenosis and insufficiency are directly applicable to aortic bicuspid valves in adults.

3. *Predominance of Lesions on the Left Side.*—The predominance of lesions on the left side of the heart has long been appreciated. The difference in pressure on the two sides has been the most logical and most apparent cause given. However, if there were such a relationship here, one might expect the ratio between the incidence of right-sided lesions and the incidence of left-sided lesions to be approximately similar to the ratio between the pressure in the pulmonary artery and the pressure in the aorta. Therefore, the incidence of right-sided bacterial endocarditis was compiled from the combined series of Blumer,¹⁹ Libman,¹⁷ Clawson,³ Horder³⁵ and Thayer.¹⁸ In a total of 859 cases, 10.3 per cent involved the right side of the heart. The pulmonary arterial pressure is about one sixth or 16.6 per cent of that of the aorta (Wiggers,³² Best and Taylor¹³). These figures—10.3 per cent and 16.6 per cent—are sufficiently close to be at least consistent with a possible relationship.

Another pertinent fact concerns the incidence of lesions on the right side in cases of acute and cases of subacute endocarditis. An analysis of Thayer's figures shows that there is a distinctly greater incidence of lesions on the right side in the cases of acute than in the cases of subacute endocarditis. Furthermore, Libman's¹⁷ statistics reveal that 26.8 per cent of 56 cases of acute bacterial endocarditis involved the right side, whereas "only 1 in over 100" cases of the subacute variety were on this side. Kaufman³⁶ also stated that endocarditis of the acute type more commonly involves the right side. Although Clawson's³ data do not confirm this conclusion, there is abundant evidence in support of it. In other words, there seems to be a generally greater tendency for the virulent rather than the relatively avirulent organisms (e. g., *Streptococcus viridans*) to involve the right side. The reason suggesting itself is that the *necessity for pressure, impact and contact diminishes as the virulence increases*. Additional evidence tending to substantiate this view may be had from Thayer's figures on preexisting valvular disease.

34. Gross, L.: Arch. Path. **23**:350, 1937.

35. Horder, T. J.: Quart. J. Med. **2**:289, 1909.

36. Kaufman, E.: Pathology for Students and Practitioners, translated by S. P. Reimann, Philadelphia, P. Blakiston's Son & Co., 1929, vol. 1, p. 30.

A preexisting deformity was found in 22 per cent of the cases of gonococcic endocarditis, 35 per cent of the cases of pneumococcic endocarditis and 45 per cent of the cases of staphylococcic endocarditis. On the other hand, such a valvular deformity was present in 82 per cent of the cases of subacute bacterial endocarditis due to streptococci. This also seems to indicate that the necessity for the deformity with the resultant changes in impact and contact diminishes as the virulence of the organism increases. That this should be so is perfectly natural and ties up with the entire concept herein advanced.

4. *Rarity of Bacterial Endocarditis in Patients with Auricular Fibrillation Secondary to Rheumatic Mitral Stenosis.*—Finally, there is the



Fig. 3.—Acute bacterial endocarditis superimposed on a rheumatic fibroplastic deformity. This occurrence illustrates the importance of the mechanism of selective impact. Note that the vegetation localized beneath the projecting shelf, which bore the brunt of impact, rather than at the line of closure, which in this case happens to be thin, flexible and able to give way with the stream.

well recognized fact that bacterial endocarditis is rarely implanted in a fibrillating heart (Libman¹⁷; Fishberg²⁷). Many equivocal explanations have been offered,^{36a} among them the correlation with the degree of mitral stenosis, the integrity of the myocardium, the grade of immunity, the short life expectancy of patients with auricular fibrillation and mitral stenosis, and finally mere chance distribution. Each of these theories

36a. Segal, M. S.: *Am. Heart J.* **11**: 309, 1936.

has been challenged, and Fishberg concluded that no adequate explanation has thus far been offered. It may be of interest, therefore, to analyze briefly the circulatory dynamics of this arrhythmia.

In auricular fibrillation, "forcible ejection into the ventricle does not occur" (Best and Taylor³⁷). By the very mechanism of irregular fibrillatory contraction, the important factor of resistance and impact against the valve is reduced to a minimum, as the blood drools through the valvular orifice. This of itself suggests a possible explanation for the failure of implantation on only the auriculoventricular valves. With regard to the semilunar valves, auricular fibrillation produces several changes in hemodynamics which reduce impact and contact with these valves:

1. Fishberg states that "the evidence is unequivocal that auricular fibrillation tends to decrease the cardiac output" (Smith and others³⁷; Kerkhof³⁸; Harrison and others³⁹).

2. The ventricular rate is generally rapid, thus impeding diastolic filling and the force of contraction.

3. Finally, contractility is often not fully restored with each impulse; therefore the valve frequently opens feebly, as indicated by the pulse deficit.

In other terms, auricular fibrillation produces a distinct reduction in the potency of impact and frictional resistance, especially at the line of closure. It is maintained that the possibilities for implantation of bacterial vegetations are thereby correspondingly reduced. One does not wish to imply, however, that other factors may not play an auxiliary role.

COMMENT

In review, it is emphasized that any theory purporting to explain the mechanism of localization of bacterial vegetations must be challenged by at least four basic clinical and pathologic facts concerning endocarditis. Each of these has been discussed separately, and throughout the discussion the same principles have been employed to account for these facts in spite of their apparent diversity. However, it must be stressed that the concept of impact and contact attempts to explain not why implantation occurs but simply why implantation occurs at a particular site. It is felt that in all probability this localization takes place after the stage has been set by a generalized altered reaction of tissue or immunity. Evidence for this is furnished not only from clinical material

37. Smith, W. C.; Walker, G. L., and Alt, H. L.: *Arch. Int. Med.* **45**:706, 1930.

38. Kerkhof, A. C.: *Am. Heart J.* **11**:206, 1936.

39. Harrison, T. R.; Friedman, B.; Clark, G., and Resnik, H.: *Arch. Int. Med.* **54**:239, 1934.

(Swift ⁴⁰; Hector ⁴¹) but from the sensitization experiments of Rosenow,⁴² Semsroth and Koch,²⁴ Freifeld,⁴³ Birkhaug,⁴⁴ Kinsella,⁴⁵ Wright ⁴⁶ and others.

SUMMARY

In from 50 to 75 per cent of cases bacterial endocarditis is superimposed on rheumatic endocarditis. Rheumatic endocarditis commonly produces a valvular fibroplastic deformity with stenosis. This lesion takes the form of a projecting shelf or barrier, usually at the line of closure, against which the blood stream strikes. By virtue of this obstruction to the systolic discharge (manifested by myocardial hypertrophy) the site of the deformity suffers a distinctly greater impact and contact than the normal valve leaflet, which "gives" or yields with the stream. This contact with the blood (and organisms in a bacteremia) is further enhanced by the diastolic backflow due to insufficiency—the usual concomitant of stenosis. The role of these dynamics in the localization of vegetations is suggested.

This same mechanism applies to congenital lesions including the "so-called congenitally bicuspid aortic valve."

Attention is called to the fact that the outflow surface of all valves, normal or deformed, comes in contact with a much greater area of blood (and toxic agents) than the opposite surface. The significance of this in the localization of vegetations is stressed.

An explanation based on the same principles is offered for the rarity with which auricular fibrillation is complicated by bacterial endocarditis. The possibility of the influence of other auxiliary factors is not precluded.

It is pointed out that there is an increased tendency for acute rather than subacute endocarditis to occur on (*a*) valves not previously deformed and (*b*) valves of the right side of the heart. This fact is correlated with the principles of impact and contact.

1 East One Hundredth Street.

40. Swift, H. F.: *Am. Heart J.* **3**:629, 1928.

41. Hector, F. J.: *Arch. Dis. Childhood* **1**:339, 1926.

42. Rosenow, E. C.: *J. Infect. Dis.* **7**:411, 1910.

43. Freifeld, H.: *Klin. Wchnschr.* **7**:1645, 1928.

44. Birkhaug, K. E.: *J. Infect. Dis.* **40**:549, 1927.

45. Kinsella, R. A., and Sherburne, E. C.: *J. A. M. A.* **80**:1643, 1923.

46. Wright, A. D.: *J. Path. & Bact.* **29**:5, 1926.

STUDIES OF HUMAN OVA

I. DESCRIPTION OF ARTIFICIALLY INDUCED PARTHENOGENETIC ACTIVITIES IN ONE

STANLEY P. REIMANN, M.D.

AND

BERNARD J. MILLER

PHILADELPHIA

The eggs of invertebrates are capable of parthenogenetic development, and many species normally multiply by this process. Not only this, but Loeb,^{1a} Allen and Bonta^{1b} and others have shown that the eggs of many species that do not normally multiply by parthenogenesis can be made to undergo parthenogenetic division by mechanical stimulation or by treatment with various organic acids and many other substances. For obvious reasons, mammalian parthenogenesis has not received as much attention. Loeb² and others have shown that parthenogenesis can take place in the ovarian eggs of the guinea pig, but there seems to have been no direct application of experimental methods to human tubal eggs for the purpose of inducing cleavage or other cellular activities. The human tubal ovum has remained uninvestigated except as to its morphologic character.

From the pathologic as well as many other points of view parthenogenesis in the human ovum is of great interest.

For many years, dermoid cysts, teratomas, certain hamartomas and other tumors have been said to have parthenogenetic origin from ova, and this origin would probably be accepted as possible by most observers if some one would only find that the human ovum can, in fact, become active parthenogenetically. In his monograph³ Bosaeus reviewed the literature and findings, detailed experiments on parthenogenetically

From the Lankenau Hospital Research Institute.

In this investigation we were assisted by a grant for research on growth from Mrs. William Nax and by special equipment from an anonymous donor.

1. (a) Loeb, J.: Univ. California Publ., Physiol. **1**:7, 1903. For discussion and literature see Morgan, T. H.: Experimental Embryology, New York, Columbia University Press, 1927, pp. 537-593. (b) Allen, E., and Bonta, A. M.: J. Morphol. **48**:123, 1929.

2. Loeb, J.: Anat. Rec. **51**:373, 1932.

3. Bosaeus, W.: Beiträge zur Kenntnis der Genese der Ovarialembryone: Experimentelle Untersuchungen über parthenogenetische Ovarialgravidität bei Amphibien, Uppsala, Almqvist & Wiksells, 1926.

developed amphibian ova and reached the deduction that the human ovum can probably become active parthenogenetically. This is now shown to be the case as described in subsequent paragraphs.

In addition, the doctrines of multiple and excessive potency are of great interest to oncologists.⁴ The potency displayed by the ordinary nonfertilized human ovum is nil; it is now apparent that the actual potency is greater than the ordinary realization of that potency, just as in all other cells in which there still remain some powers of differentiation.

Details sufficiently accurate for these experiments of the ovulatory period in the human female are sketchily known. Allen, Pratt, Bland and Newell,⁵ by a study of five human tubal ova recovered on the fourteenth, fifteenth and sixteenth days and a study of the appearance of the follicles from which these ova were discharged, set the period of ovulation as between the eleventh and the thirteenth day after the onset of the last menses. With a potentiometric device Burr and Musselman⁶ recorded a sharp increase in potential in the human female on the fifteenth day before the onset of the next menses, presumably making the period of ovulation the thirteenth day after the onset of the previous menses.

The best chance of obtaining freshly discharged ova seems to be within this period, although one of our ova was obtained on the twenty-fourth day after the onset of the last menses.

To date (Aug. 15, 1938) five have been recovered. Numerous observations have been made, which will be published when additional specimens have been obtained to clarify certain points, such as density of capsule and accumulation of albumin around the ovum. Meanwhile, the behavior of the one ovum which became parthenogenetically active on "stimulation" will be recorded. None of the other four showed any spontaneous changes like it; nor can we find any mention of such changes in human ova by a review of the literature or by personal questions.

METHOD

Through the cooperation of the surgical staff, selected patients scheduled for hysterectomy were submitted to operation on the fifteenth or the sixteenth day after the onset of the last menses. In some cases the specimen consisted of

4. Reimann, S. P.: *Biology of the Cancer Cell*, in a Symposium on Cancer: Addresses Given at an Institute on Cancer Conducted by the Medical School of the University of Wisconsin, Madison, Wis., University of Wisconsin Press, 1938, pp. 114-134.

5. Allen, E.; Pratt, J. P.; Bland, L. J., and Newell, Q. U.: *Human Tubal Ova; Related Early Corpora Lutea and Uterine Tubes*, Publication 414, Carnegie Institution of Washington, 1930; *Contrib. Embryol.* **22**:45-76, 1930.

6. Burr, H. S., and Musselman, L. K.: *Yale J. Biol. & Med.* **9**:155, 1936.

the uterus, both tubes and both ovaries; in some cases only one tube was removed. Regardless of the kind of specimen, as soon as possible after it was removed, the tubes were cut from the uterus. A no. 20 Luer type needle was inserted into the fimbriated end and 10 cc. of Locke's solution was gently forced through. The washings were collected in watch crystals. Ova rapidly settle to the deepest portion. Photomicrographs of the ova were taken at any and every stage which seemed interesting by a Leica micro camera attachment. In some cases the ova were transferred to a hanging drop of human serum and then placed on the warm stage of a microdissecting apparatus. In other cases the original watch glass was used. But further details of this will be published with the observations on the respective ova, together with experiences in the use of rabbit and rat ova.

DESCRIPTION OF PARTICULAR OVUM

The specimen was obtained July 18, 1938, from a colored woman aged 35 years, whose periods averaged twenty-eight days. The flow lasted usually five days and was profuse. On the fifteenth day after the onset of her last menses, a specimen was removed at operation, consisting of two fallopian tubes, a fibroleiomatous uterus and one ovary. The ovary was soft and pink-gray, and had been partially resected at a previous operation. The other ovary was not removed.

The tubes were cut from the uterus and then washed with Locke's solution into watch crystals. The ovum was identified under low power and transferred to a hanging drop of human blood serum to which had been added a droplet of ethyl ester of acetic acid. The preparation was then placed in a warm, moist chamber.

The ovum on first appearance was rather large and quite uniform (fig. 1 *A*). On closer observation, it was found to be expanded against the internal surface of the zona pellucida. The zona pellucida was clear and uniform, appearing as a shell totally investing the contained vitellus. There was no perivitelline space since the ovum was everywhere in contact with the zona pellucida. At one point on the peripheral margin of the vitellus, directly beneath the zona pellucida, there was a marginal darkness which appeared to be a polar body. When the ovum was rolled over on the opposite side, this body became more evident. On focusing at different levels a darker central portion was seen. There were no vacuoles, and as far as observation could determine the ovum appeared fresh. The cytoplasm was not uniform in composition, and thus the ovum was slightly older than other specimens. (In younger specimens the cytoplasm is said to be more homogeneous.) Various-sized yolk granules were seen, and variations in the color of these granules were also observed.

The cytoplasm of the ovum was largely composed of small yolk granules. These granules were quite translucent to light and were tinged faintly yellow. The larger granules were more opaque to light and were more deeply colored. One large chromophoric body was

observed near the margin of the ovum. This body appeared to have radiating strands directed toward the zona pellucida and toward the central portion. The largest strand was directed toward the zona pellucida while the smaller strands were directed toward the central portion of the ovum. The dark yellow granules were but four in number and were evenly distributed throughout the ovum.

Within a half hour after being first seen, the ovum was transferred to a hanging drop of freshly drawn blood serum, to which had been

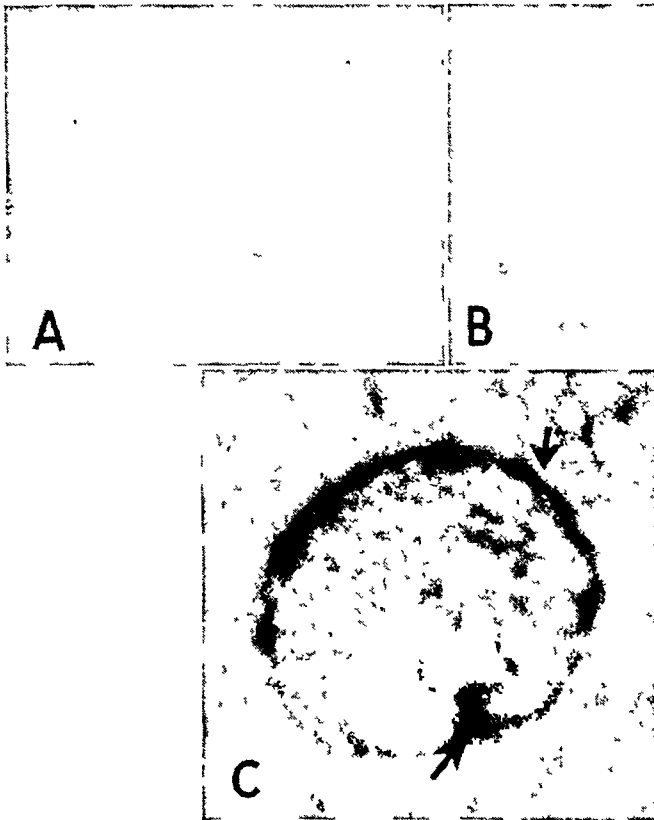


Fig. 1.—*A*, first appearance of the ovum, 89.6 microns in diameter; *B*, ovum rolled over on the other side to show chromophoric body; *C*, ovum rolled over with chromophoric body almost out of view to show furrow more distinctly; 4 mm. objective $\times 10$ periplan. ocular; tube length, 160 mm.; photographic enlargement, $\times 11$.

added a droplet of ethyl ester of acetic acid. This is known to stimulate parthenogenetic development in lower forms (Loeb). In transferring the ovum from Locke's solution a very small amount of calcium ion was necessarily introduced into the medium. The moist chamber was placed on the warm stage of the microdissecting apparatus, and both needles were brought into the field. The right hand needle was then brought to bear against the ovum and slight force exerted. The zona pellucida

offered some resistance to the entrance of the needle. As the needle was forced against the zona, a small indentation was produced, which disappeared just as soon as the needle was withdrawn. This seems to indicate that the zona pellucida is slightly elastic but tough. When more force was exerted on the vertical control, the needle entered the ovum very quickly. This jerky entrance into the ovum likewise indicates that the zona pellucida is tough.⁷ On entrance the needle encountered no difficulty in further progress.

The needle was quickly removed from the ovum. Twenty minutes after the puncture the ovum underwent a definite cytoplasmic change. The zona pellucida became considerably thinner at one pole, while the

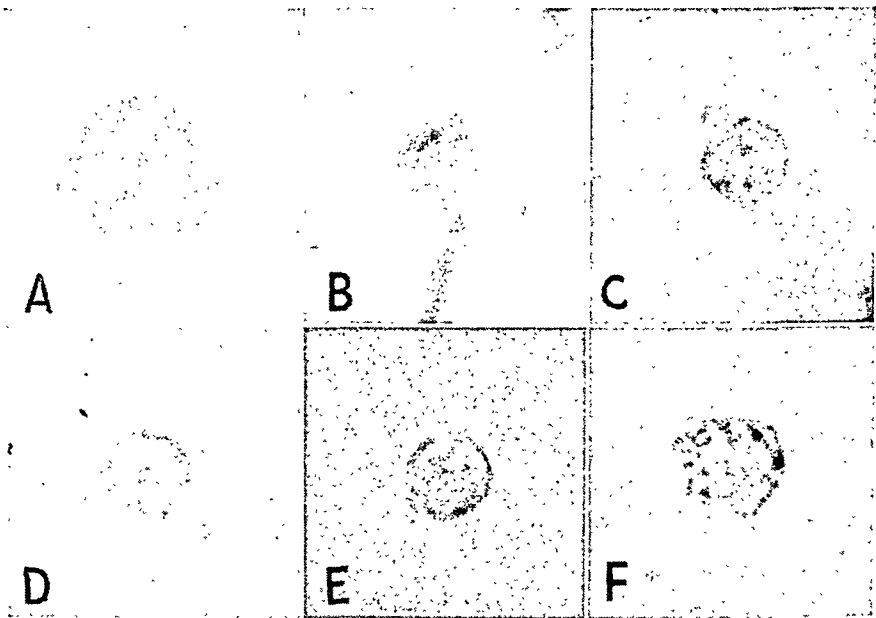


Fig. 2.—*A*, ovum when first transferred to microdissecting apparatus; *B*, ovum ten minutes after puncture; *C*, first extrusion, with crater in ovum; *D*, beginning of second extrusion; *E*, second extrusion; *F*, last stages in disintegration.

cytoplasm protruded directly beneath the now thinner zona pellucida. In about five minutes this cytoplasmic pseudopod had pushed itself out beyond the peripheral margin of the ovum and remained attached by a stalk, visible in figure 2 *B*. The base of the stalk then became constricted and in a very short time detached itself and assumed a spherical shape. Within a few minutes the small polar body migrated to a distance from the ovum (fig. 2 *C*).

The extrusion of the body left the ovum altered in appearance. That portion which had previously extruded the polar body now appeared as a

7. Dr. Georg S. de Renyi, in a personal communication, states that he has observed similar toughness in the rabbit's ovum.

very deep crater with a rough margin (fig. 2 *C*). Within five minutes the crater disappeared, and the ovum remolded itself. After another twenty minutes the ovum again showed signs of similar activity. A small peripheral granule again appeared directly beneath the zona pellucida (fig. 2 *D*). Although a bit slower than the first, the second body was also cast free from the ovum in about twenty-five minutes (fig. 2 *E*). After the extrusion of the second polar body, the ovum again showed a crater but one not quite as deep as the first one (fig. 2 *E*). The second polar body was a bit larger than the first but except for this difference was quite similar in appearance. After the extrusion of the second polar body, the ovum began showing changes that were undoubtedly stages in disintegration of the cell. Large peripheral vacuoles appeared in the region from which the polar bodies had been extruded, and the cytoplasm became increasingly more granular. The zona pellucida remained intact on the side that was not involved in the extrusion of the polar bodies. In figure 2 *F* the cell is shown about five hours old, in the final stages of disintegration.

During the course of this activity, another phenomenon was observed, which obviously could not be investigated in a stained preparation without sacrificing the other observations. A furrow was formed in the vitellus beginning from the dark chromophoric body previously mentioned (fig. 1 *C*). On one side the furrow was incomplete, but when the ovum was gently rolled over on its other side the furrow was seen as a moderately deep crater traversing the entire ovum (fig. 1 *D*). During the extrusion of the polar body the furrow increased in size but little. Its appearance was exactly that of the cleavage furrow seen in tissue culture cells. It is interpreted as the beginning of cleavage. More experience with other human ova, as well as with ova of other mammals, in relation to mediums, temperature and other factors have now made it possible to keep ova in better condition over greater lengths of time. Further experiments will probably lead to more complete cleavage, such as we have seen in a recent experiment in which we used the same method with a rabbit's ovum.

COMMENT

Polar body fragmentation of the type just described has been observed in the unfertilized ova of the mouse and rabbit.⁸ According to Pincus, this type of activity represents a very irregular type of segmentation.⁹ As far as we can discover, it has never been observed as a spontaneous process in the human tubal ovum, nor has this process ever before been artificially stimulated in any mammal's ovum.

8. Pincus, G. G.: *Proc. Roy. Soc., London*, s.B **107**:132, 1930.

9. Personal communication to the authors.

Further, according to Pincus, polar body extrusion of this type is characterized by the formation of a deep crater. This we have clearly observed in the extrusion of both polar bodies. Thus when the division of the ovum into polar body and oocyte is about to take place, the spindle is arranged so that one pole is incorporated in that part of the ovum that is destined to be the polar body and the other part of the spindle remains eccentrically placed in the ovum. When division finally takes place, the pole of the spindle remaining in the ovum is rapidly pulled down into the central portion, causing the peripheral part of the ovum that was involved in the extrusion to be pulled down with it. This forms the crater.⁹ For this reason we feel certain that the parthenogenetic activity of this ovum was truly that in which polar bodies are extruded. On the other hand, conclusive demonstration of mitosis is best obtained from a specimen which has been fixed and stained, procedures which, as stated, could not be carried out without sacrificing further observation. But other ova will be prepared in this manner, and the results will be reported in a later paper. Further reasons are that the activity could not have been a surface precipitation reaction, because if it had been due, for example, to the presence of a small amount of calcium ion it would have taken place many times faster than it did.¹⁰ Then, the second polar body was extruded without any mechanical stimulation. The ovum was initially stimulated, and this process followed minutes after.

SUMMARY AND CONCLUSIONS

Five unfertilized human tubal ova have been recovered from fallopian tubes of patients operated on for fibroleiomyoma and other noninflammatory pelvic conditions especially on the fifteenth and sixteenth days after the onset of the last menses.

Parthenogenetic activities resulting in extrusion of polar bodies and the formation of a deep cleft in the oocyte are described as they occurred in one particular unfertilized tubal ovum after mechanical stimulation in a medium of human blood serum containing a minute trace of the ethyl ester of acetic acid. The details of observations and experiments with four others will be published later, when a number of additional specimens have been studied to clarify certain special points.

It is concluded that the human ovum is capable of being artificially stimulated to parthenogenetic activity.

The implications of this finding are many and varied. There are mentioned but two: its relation to dermoids, teratomas, etc., and its bearing on concepts of potency.

10. Heilbrunn, L. V.: *General Physiology*, Philadelphia, W. B. Saunders Company, 1937, pp. 79, 81 and 542-546.

RARE LOCALIZATIONS OF HYDATID DISEASE IN TURKEY

REPORT OF A CASE OF PLURIVISCERAL ECHINOCOCCOSIS

PERIHAN ÇAMBEL, M.D.

ISTANBUL, TURKEY

Echinococcosis is not a rare condition in Turkey. The parasite that is usually met with is *Echinococcus hydatidosus*. No infection with *Echinococcus alveolaris* has been observed in Turkey that I know of. Hydatid disease in this as in other countries is usually seen most frequently in the liver and in the lungs. Rare localizations have been the subject of several publications. Içgören,¹ in a case of hydatid cyst of the left lung, observed roentgenologically a cyst of the left tibia with a fistula which had been caused by trauma. Microscopic examination of the contents of the cyst in the tibia proved the cyst to be due to the echinococcus.

Schükrü-Aksel² at a postmortem examination observed primary hydatid cysts in the spleen, which weighed 10 Kg., and in the mesentery. Neither the liver nor the lungs showed any involvement. In the discussion of this case Avni Aksel mentioned the observation of a young boy who showed generalized echinococcosis of the peritoneal cavity after an operation for appendicitis; about 40 hydatid cysts were removed. Exploration of the liver and the spleen of this patient showed multiple cysts in both organs. Lutfi Aksu mentioned a necropsy in which generalized peritoneal echinococcosis was observed.

F. Kâmil (cited by Çetingil³) and Çetingil³ each published a case of echinococcosis involving the kidney. In the case observed by the latter, hooks and leukocytes were seen in microscopic examination of the urine and of the fluid obtained by puncture of the pleura. On the sixth day of the patient's stay at the clinic he vomited 200 cc. of pus containing leukocytes and hooks. Even then no evidence of a cyst could be seen in any part of the lungs. The cyst of the kidney had perforated through the diaphragm into the lung. The patient was sent to the

From the Laboratory for Pathological Anatomy, Gureba Hospital.

1. Içgören, K. N.: *Türk tib cem. mec.* **1**:517, 1935.

2. Schükrü-Aksel, I.: *Türk tib cem. mec.* **2**:38, 1936.

3. Çetingil, A. I.: *Türk tib cem. mec.* **2**:61, 1936.

urologic clinic for nephrectomy. Nissen⁴ observed periodic appearances of hydatid cysts in the urine. The right kidney of his patient was removed. The same author,⁵ while operating on a patient for periodic obstruction of the common bile duct and cholangitis, had to remove multiple cysts after choledochotomy. A big echinococcic cyst of the convex face of the liver had been originating them.

H. Hamdi and Tevfik Saglam reported a case of primary hydatid cyst of the heart, and Hazim Bumin,⁶ a case of primary hydatid cyst of the left breast in a woman 25 years of age. In the Hamdi Museum of the Gureba hospital there is a hydatid cyst, 8 cm. in diameter, in a liver with atrophic cirrhosis.

It appears from this account that there may be primary or secondary involvement of organs other than the liver or the lungs. It is an accepted fact that the "hexacanth embryo" has to pass the first filter (liver) and then the second filter (lungs) before it can penetrate into the great arterial circulation. According to Sabadini,⁷ the most frequent localizations are in the liver (75 per cent) and the second most frequent are in the lungs (10 per cent). This leaves only 15 per cent of the cases in which other organs, such as those mentioned in the foregoing review, are involved.

REPORT OF CASE

A 53 year old miller, born in Pristine, Albania, had been admitted to the second medical clinic of the University of Istanbul, Nov. 4, 1937. An exploratory laparotomy disclosed peritoneal echinococcosis. Removal of the cysts proved to be impossible. It was decided, also, that certain tumoral elevations of the diaphragm seen roentgenologically were hydatid cysts. The patient died April 29, 1938.

Necropsy.—The body was that of a hairy man, 170 cm. in length and cachectic. The abdomen was extremely swollen. The umbilicus was obliterated by the scar of an operation—a scar 15 cm. long, beginning in the epigastric region. When the abdominal cavity was opened, implantation cysts were seen between the skin and the rectus muscles and between the muscles and the peritoneum (fig. 1 S). The peritoneum contained about 500 cc. of a clear yellowish fluid and was filled with multiple cysts, adhering partly to one another and partly to the abdominal wall. The adhesions were usually so firm that the cysts could be separated only by the knife. It was impossible to separate those of the abdominal wall from those of the liver. Still the bile ducts were absolutely free and the bowels colored. The intestinal loops were almost loaded with cysts, and the small pelvis was filled with them. Nowhere was there a sign of intestinal obstruction. Here and there thin walled cysts, pedunculated, were hanging free in the peritoneal cavity. Some of the cysts consisted simply of mother cysts; others contained daughter and granddaughter cysts. A great cystic tumor, almost

4. Nissen, R.: *Türk tib cem. mec.* 2:464, 1936.

5. Nissen, R.: *Türk tib cem. mec.* 2:507, 1936.

6. Bumin, Hazim: *Türk tib cem. mec.* 4:262, 1938.

7. Sabadini, L.: *Les kystes hydatiques de la rate*, Paris, Masson & Cie, 1936.

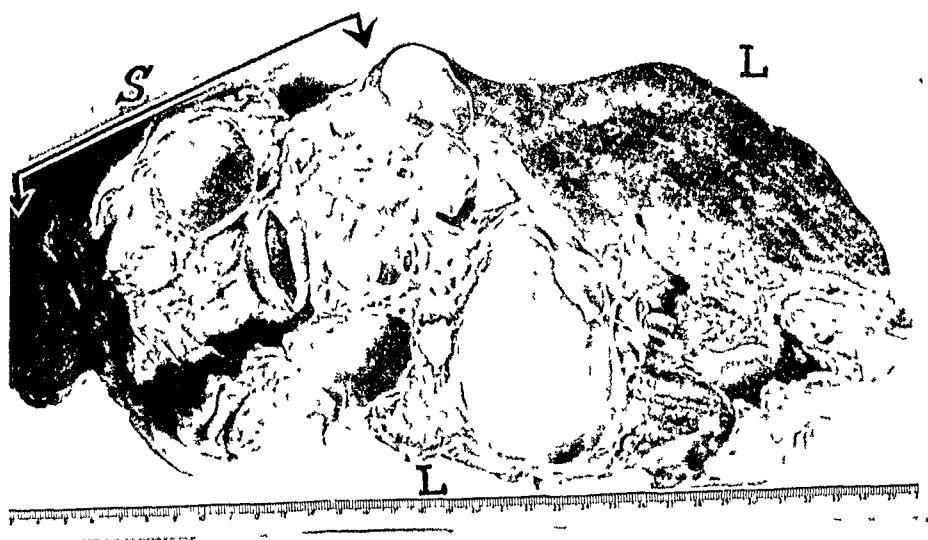


Fig. 1.—Hydatid cysts revealed in section of the liver, *L*. Note the bulk of cysts adherent to the lower surface of the liver and the abdominal wall, *S*.



Fig. 2.—Mass of peritoneal implantation cysts. *A* designates the appendix.

filling the left half of the abdominal cavity, proved to be the spleen. The diaphragm showed cystic involvement on both sides and was pushed up by the liver and spleen.

The liver with the cysts adhering to it weighed 5,220 Gm. The parenchyma of the liver contained several large cysts and was greatly reduced.

The spleen weighed 1,820 Gm. and contained equally numerous hydatid cysts, lined in part by the splenic tissue, which was increased.

The appendix had been obliterated by small cysts. The other abdominal organs were not involved.

The heart showed general hypertrophy and slight dilatation, especially of the *conus arteriosus*.

Both lungs adhered firmly to the parietal pleura. The right lung contained two sacciform bronchiectases toward its apex. In the upper lobe of the left lung a cavity almost the size of a Maltese orange was observed, which contained dirty greenish pus and drained into a bronchus. The pulmonary tissue surrounding it was infiltrated.

No localizations were found in the bones. There was general hydremia.

Microscopic Examination.—The clear colorless liquid of the cysts was centrifuged and found to contain rare hooks.

The cyst walls were constructed of rarely cellular, mostly collagenous connective tissue, showing inflammatory infiltrations of small lymphocytes and histiocytes. Inside of the outer cystic wall the inner cystic wall, consisting of concentric layers of chitin, formed many folds. No eosinophils were observed. It is interesting to note that their absence coincided with negativity of the Cazoni test; hence the patient must have been in a nonallergic state.

The wall of the abscess in the lung was of the same structure as the outer connective tissue walls of the cysts, so that it may be concluded that the abscess cavity was the remnant cavity of a hydatid cyst drained by the bronchus and suppurating secondarily. The content of the surrounding alveolar spaces was partly plasma and partly alveolar cells. The alveolar walls had been thickened by an increase in connective tissue. Some alveolar spaces had even been completely obliterated.

The liver cells had stored lipofuscin. Their protoplasm was granular. The spaces between them had enlarged. The reticular cells were edematous. In some places the chains formed by the liver cells were loosened, and single cells, isolated from one another, were to be seen. These alterations could be taken as preliminary signs of toxic hepatitis.

The spleen showed signs of fibrosis and stasis.

COMMENT

It is said that the frequency of hydatid cyst of the spleen shows geographic variations. Involvement of this organ is rarest in Iceland (according to Finsen, the incidence is 0.78 per cent) though in that country hydatid disease is of great frequency. Hydatid disease of the spleen is said to be more frequent in Russia than in Germany or France. The greatest incidence of the condition is said to be observed in Italy and Algiers, Algeria (4, 6 and 10 per cent, according to different authors). It is doubtful whether these figures have the significance of absolute values. In Turkey hydatid cyst of the spleen seems to be a relatively rare form of echinococcosis.

The first report of a case of hydatid disease of the spleen was published by Berthelot (1790). Sabadini⁷ in his excellent monograph on this subject cited 288 cases that had occurred since that date. Among reports of more than 200 observations consulted, mention was made of only 10 instances in which the spleen presented a multiplicity of cysts. In 6 of these the spleen contained only two cysts; in the others, three, four and six. In the spleen described here twelve cysts of different sizes were counted, which shows it to be a rare specimen.

According to Dévé, a massive arrival of cysticerci in the splenic parenchyma is very rare. Sabadini proposed two pathogenic possibilities: (1) the passage of several cysticerci through the hepatopulmonary filters; (2) an exogenous vesiculation originating from the primordial vesicle. The first possibility has been accepted by many authors.

Splenic hydatid disease may be associated with other visceral localizations, as in the liver, the peritoneum or the liver and the peritoneum. The peritoneal involvement is secondary to perforation of hepatic or splenic cysts. Therefore the peritoneal cysts are called metastatic implantations.

The rarest type of plurivisceral localization seems to be simultaneous involvement of the lungs and spleen (Michon; Angelescu; Verequunov; Hermann—all cited by Sabadini⁷). Goyrand-d'Aix observed coexistence of splenic and pulmonary cysts with a cyst of the ligamentum latum uteri. Martin observed four cysts of the spleen, one of the lung and five of the omentum.

It is clear that the extent of the peritoneal generalization, the polycystic obliteration of the appendix, the multiple diaphragmatic, hepatic and splenic cysts coexisting with a drained but secondarily suppurating pulmonary cystic cavity make my case an instance of one of the rare types of plurivisceral localization. Concerning the splenic cysts, I believe that they resulted after the passage of cysticerci across the two filters. Concerning the peritoneal cysts, I think that they may have originated in two different ways: The first might have been by perforation from the liver and possibly also from the spleen, after which the cysticerci would have become implanted on the peritoneum next to these organs. As new vesicles were found hanging free in the peritoneal cavity, I believe that the aforementioned implantations gave rise, in turn, to exogenous growth of these vesicles.

SUMMARY

A short review has been made of cases of rare localizations of hydatid disease as recorded in Turkey. A rare case of plurivisceral echinococcosis involving the liver, diaphragm, left lung, spleen, peritoneum and appendix is reported and discussed.

EXPERIMENTAL ENDOCARDITIS IN DOGS

MARGERY BLAHD, M.D.

IRA FRANK, M.D.

AND

OTTO SAPHIR, M.D.

CHICAGO

Experimental endocarditis and its relation to the genesis of the disease in man has for more than fifty years been a problem not satisfactorily solved. The subject was brought to our attention when we encountered, quite accidentally, acute endocarditis in a dog following an intravenous injection of streptococci.

In experiments designed to produce meningitis, we were attempting to increase the virulence of a strain of beta hemolytic streptococcus by passing it through the blood stream of dogs. At autopsy of one of these animals we incidentally found vegetative endocarditis. Realizing that experimental endocarditis, despite much investigation, remains a matter of considerable confusion, we became interested in it.

Because much new work has been done in this field, it may be advisable to discuss briefly the present conception of the genesis of experimental endocarditis. According to the literature, positive results have been obtained by four general methods: (1) mechanical procedures with intravenous introduction of virulent bacteria, (2) simple intravenous injection of bacteria, (3) production of a bacterial focus in the body and (4) preliminary injection of predisposing substances, with subsequent intravenous injection of virulent organisms.

In the earliest instances experimental endocarditis was produced by mechanical means.¹ One method consisted in producing injuries of the valves with sharp instruments introduced through the great vessels and then injecting virulent organisms intravenously. Using this procedure, Kinsella and Hayes² produced endocarditis regularly. Another mechanical method was the intravenous injection of bacteria in mediums composed of large particles, such as pulverized carbon,³ potatoes,⁴ emulsion

From the Department of Pathology of the Nelson Morris Institute, Michael Reese Hospital.

1. Rosenbach, O.: *Arch. f. exper. Path. u. Pharmakol.* **9**:1, 1878.

2. Kinsella, R., and Hayes, C.: *Proc. Soc. Exper. Biol. & Med.* **24**:887, 1927.

3. Fulci, F.: *Beitr. z. path. Anat. u. z. allg. Path.* **44**:349, 1908.

4. Ribbert, cited by Lissauer, M.: *Centralbl. f. allg. Path. u. path. Anat.* **23**: 243, 1912.

of carcinomatous cells⁵ or sterile flour.⁶ Bonome⁷ used cultures of staphylococci which grew in large clumps. He assumed that bacterial emboli, thus formed, caused the endocarditis.

The reliability of the simple intravenous injection of bacteria in the production of endocarditis varies in the hands of different investigators. Although sporadic positive results have been obtained, there is agreement among most workers in the field that this method is entirely undependable. On the other hand, Rosenow⁸ reported endocardial lesions in 84 per cent of 44 rabbits receiving intravenous injections of large numbers of organisms isolated from patients with subacute bacterial endocarditis. As a matter of fact, most of the positive experimental results have been produced in rabbits; production of endocarditis in dogs by the simple intravenous method has been reported in very few instances. Lanfranchi⁹ produced endocarditis in a dog by a single injection of staphylococci, and with two injections, administered four weeks apart, reproduced endocarditis in another dog. Fox¹⁰ reported positive results in 3 dogs on injection of streptococci secured from patients with rheumatic fever and puerperal sepsis. Cornil, Mosinger and Haimovici¹¹ were able to produce endocarditis in dogs by a simple intravenous injection of streptococci isolated from patients with endocarditis, but they supplied no figures.

The experiments of Welch, Murdock and Ferguson¹² illustrate the third general method for the production of experimental endocarditis: They planted foci of *Streptococcus viridans* in teeth of rabbits, needled the hearts of the rabbits and sprayed the throats with influenza bacilli. Friedman, Katz and Howell,¹³ using dogs, established a bacterial focus directly in the cardiac cavity by inserting through the thoracic wall a bakelite capsule containing blood agar cultures of *Str. viridans*. By this device they produced endocarditis. Birkhaug¹⁴ reported the production of endocarditis in rabbits by introducing intramuscularly a focus

5. Panichi, L., and Guelfi, C.: *Virchows Arch. f. path. Anat.* **198**:449, 1909.

6. Viti, cited by Lissauer, M.: *Centralbl. f. allg. Path. u. path. Anat.* **23**:243, 1912.

7. Bonome, A.: *Arch. ital. de biol.* **8**:10, 1887.

8. Rosenow, E. C.: *J. A. M. A.* **65**:1687, 1915.

9. Lanfranchi, cited by Rievel, H.: *Ergebn. d. allg. Path. u. path. Anat.* **17**:2, 1915.

10. Fox, H.: *Centralbl. f. allg. Path. u. path. Anat.* **24**:529, 1913.

11. Cornil, L.; Mosinger, M., and Haimovici, H.: *Compt. rend. Soc. de biol.* **122**:685, 1936.

12. Welch, H.; Murdock, T., and Ferguson, J.: *J. Lab. & Clin. Med.* **21**:1264, 1936.

13. Friedman, M.; Katz, L. N., and Howell, K.: *Arch. Int. Med.* **61**:95, 1938.

14. Birkhaug, K.: *J. Infect. Dis.* **40**:549, 1927.

of bacteria in blood agar and subsequently injecting the same organisms intravenously.

Recently much attention has been focused on the procedure which includes a preparatory injection of a substance thought to effect a predisposition to the development of endocarditis in the animal organism or in the valve tissues themselves. This preliminary injection of vaccine, casein or other substances usually administered intravenously, is followed after a varying period of time by an intravenous injection of virulent bacteria.

The efficacy of preparatory injections of specific vaccines has been investigated widely. The results have varied too much to be accepted without question. After establishing that intravenous injections of living cultures of streptococci always produced septicemia in normal rabbits, Cowan¹⁵ did obtain endocarditis in 4 of 37 animals which had been previously immunized by nonlethal doses of living streptococci administered intravenously and subcutaneously. By this method, other investigators also occasionally obtained successful results. Dietrich,¹⁶ for example, was able to produce endocardial lesions with staphylococci in 17 of 24 vaccine-treated dogs; Mair,¹⁷ using pneumococci, observed positive results in 7 of 10 immunized rabbits. On the other hand, in two series of 31 immunized rabbits Wright,¹⁸ working with living cultures of pneumococci, obtained endocarditis in only 1 rabbit.

Freifeld¹⁹ demonstrated that the preliminary immunizing injections might also be nonspecific. Eight rabbits were prepared with streptococcus vaccine and then given injections of living cultures of *Staphylococcus aureus*. In 5 of these rabbits endocarditis developed. Silberberg²⁰ produced endocarditis in each of 5 animals, but Thomson²¹ in only 2 of 12, both authors using colloidal dyes as preparatory substances and then injecting staphylococci. Other preparatory materials which have afforded some successful results are horse serum,²² toxins,²³ epinephrine²⁴ and notably casein.²⁵

Three noteworthy hypotheses have been offered in explanation of the effectiveness of the predisposing agent in the production of endocarditis.

15. Cowan, M.: *Brit. J. Exper. Path.* **5**:226, 1924.

16. Dietrich, A.: *Ztschr. f. d. ges. exper. Med.* **50**:85, 1926.

17. Mair, W.: *J. Path. & Bact.* **26**:426, 1923.

18. Wright, H. D.: *J. Path. & Bact.* **29**:5, 1926.

19. Freifeld, H.: *Klin. Wchnschr.* **7**:1645, 1928.

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One holds that a certain degree of immunity must be produced in the experimental animal. Some authorities explain in this way the efficacy of preliminary treatment with specific vaccines. Wright¹⁸ suggested that a local lesion on a valve can occur only in the presence of an immunity-inhibiting generalized infection of the body.

Another theory maintains that endocarditis results from the impairment of the reticuloendothelial function of the valvular tissue brought about by the vaccine, dyes, casein and other preparatory substances. In animals that had received intravenous injections of colloidal dyes,



Fig. 1 (dog 20).—Acute endocarditis of the mitral valve. Note the large vegetations.

histologic examination of the cells in the valves revealed droplets of the dye. This is interesting in the light of Silberberg's²⁰ tissue culture experiments which showed that macrophages containing lithium carmine are impeded in their phagocytosis of organisms. Pfuhl²⁶ described pigmentation, vacuolation and destruction of histiocytes in the cardiac valves of animals treated with vaccines and casein. Semsroth and Koch²⁷ suggested that there may be a disturbance of the detoxifying ability of the valve tissue.

26. Pfuhl, W.: *Ztschr. f. mikr.-anat. Forsch.* **17**:1, 1929.

27. Semsroth, K., and Koch, R.: *Arch. Path.* **10**:869, 1930.

The third hypothesis is that the predisposition effected by the various preparatory substances consists in an activation (creation of a resorptive function)¹⁶ or a sensitization²¹ of the valve tissues and that the establishment of such a state is a prerequisite for the development of endocarditis. This explanation arose from histologic study of the valve tissues in animals given intravenous injections of the various preparatory substances only. The principal changes noted in these studies have been a definite increase in the size and number of the cells in the valves, appearance of histiocytes and edema of the valve tissue. On the other hand, Semsroth and Koch²⁷ were unable to confirm the reports of these histologic alterations of the valves in animals given injections of vaccine and casein.

From the foregoing review it can readily be seen that there is diversity in both the experimental and the theoretic approach to the



Fig. 2 (dog 5).—Section through the tricuspid valve. Note the hemorrhages and inflammatory cells. Hematoxylin-eosin preparations; $\times 110$.

problem of experimental endocarditis, particularly in respect to the preparatory treatment. Because we found, quite by chance, endocarditis in a dog given an injection of streptococci obtained from another dog, we attempted to determine whether any preparatory treatment is, after all, an essential prerequisite to positive results; i. e., whether we could not achieve comparable results by the simple intravenous introduction of organisms a priori pathogenic for a dog.

METHODS

The organism we used was a beta hemolytic streptococcus isolated from a pneumonic lesion in a dog. A culture of streptococcus was sent to us by Dr. I. Pilot. The culture was in the smooth phase on blood agar. The organism fermented lactose and salicin but not mannitol or sorbitol. There was slight acid formation

in the presence of trehalose. The organism was a streptococcus of group C, according to the Lancefield precipitin test.²⁸

Eighteen to twenty-four hour cultures of the streptococcus in beef heart dextrose broth were administered, the number of organisms per cubic centimeter of culture averaging between 1,500,000,000 and 2,000,000,000. The culture material was always thoroughly shaken to avoid embolic accidents. The bacteria were constantly passed through dogs. Occasionally we used twenty-four or forty-eight hour blood cultures (dextrose broth), positive for hemolytic streptococci, secured from other dogs given injections of our organisms.

The dogs were adult animals of the usual variety supplied to the laboratory. Injections were made into the superficial veins of the forelegs. Blood cultures were made when indicated.

After the death of an animal a postmortem examination was made as soon as possible. At this time cultures were taken from the heart blood in dextrose broth. Microscopic sections were made from the cardiac valves, myocardium and kidneys. Routine hematoxylin and eosin staining was done.

RESULTS

The accompanying table gives the results obtained in a series of 25 animals treated in the manner described. All of these subjects died spontaneously, apparently as the result of the injection of cultures of the streptococcus.

The table shows that unquestionable endocarditis occurred in 10 of the 25 dogs (40 per cent). In 8 of these, there was definite gross endocardial involvement. In 2 dogs only hemorrhagic foci were seen on the valve at autopsy, but subsequently histologic examination disclosed definite inflammatory changes. In 4 dogs endocarditis followed a single injection.

Of the other 15 dogs, 11 revealed bronchopneumonia or pyemic lesions, while the death of the remaining 4 dogs must be ascribed to bacteremia (or septicemia) alone. Blood cultures were positive for hemolytic streptococci during the course of injections as well as at autopsy.

The virulence of the bacteria varied during the fifteen month course of the experiments. The animals which were used in "stepping up" this virulence and which were therefore given injections of substandard organisms were not included in the series.

COMMENT

By simple intravenous injection of hemolytic streptococci cultured from a pneumonic lesion in a dog, endocarditis was produced in 40 per cent of 25 dogs. It is noteworthy that in 4 dogs endocarditis was produced after a single injection. The quantity of broth injected into

28. Topley, W., and Wilson, G.: *The Principles of Bacteriology and Immunity*, ed. 2, Baltimore, William Wood & Company, 1936.

Experimental Endocarditis in Dogs

Dog	Intravenous Injections	Amount of Each Injection, Cc.	Interval Between Last Injection and Death	Changes in Endocardium	Comment
1	4 in 4 days	12	2 days	No changes	Bacteremia
2	4 in 4 days	15	4 days	Acute verrucous endocarditis of mitral valve	Slight granularity; small foci of polymorphonuclear leukocytes with deposits of fibrin
3	1	25	2 days	Acute verrucous endocarditis of mitral valve	Slight granularity; polymorphonuclear leukocytes, scattered throughout valve; increase in mononuclear cells; edema; focal myocarditis
4	1	1.5	4 days	Acute ulcerative endocarditis of mitral valve	Acute myocarditis; multiple infarcts of spleen and kidneys
5	1	2	5 days	Acute endocarditis of tricuspid valve	Hemorrhagic discoloration; polymorphonuclear leukocytes, fibrin and edema
6	1	20	7 days	Acute endocarditis of tricuspid valve	Hemorrhagic discoloration; polymorphonuclear leukocytes, numerous mononuclear cells and fibrin; acute and subacute interstitial nephritis
7	3 in 4 days	14	10 days	No changes	Pyemia (abscess in muscles of chest)
8	4 in 5 days	16	1 day	No changes	Bacteremia
9	1	6 (sediment)	20 hours	No changes	Bacteremia
10	1	6 (sediment)	12 days	No changes	Bronchopneumonia
11	6 in 7 days	26	1 day	No changes	Pyemia (abscess in muscles of forelegs)
12	4 in 5 days	18	1 day	No changes	Bronchopneumonia
13	9 in 12 days	17	1 day	Acute ulcerative endocarditis	Lesion in wall of sinus of Valsalva
14	6 in 8 days	18	1 day	Acute thrombo-ulcerative endocarditis	Lesion in wall of sinus of Valsalva
15	3 in 3 days	11	1 day	No changes	Bronchopneumonia
16	3 in 4 days	6	2 days	No changes	Bronchopneumonia
17	4 in 4 days	12	3 days	Acute vegetative endocarditis of aortic valve	Myocarditis; infarcts of spleen and kidney
18	7 in 14 days	13	3 days	No changes	Pyemia (abscess in muscles of forelegs)
19	5 in 5 days	14	2 days	No changes	Bronchopneumonia
20	8 in 9 days	21	3 days	Acute vegetative endocarditis of mitral valve	Infarcts of spleen and kidneys
21	3 in 3 days	18	3 days	No changes	Bacteremia; acute focal glomerulonephritis
22	7 in 10 days	15	3 days	Acute vegetative endocarditis of tricuspid valve	Myocarditis
23	5 in 9 days	20	2 days	No changes	Bronchopneumonia
24	5 in 5 days	26	2 days	No changes	Bronchopneumonia
25	6 in 7 days	16	1 day	No changes	Pyemia (abscess in kidneys)

these 4 animals varied so greatly (from 1.5 to 25 cc.) that the number of the introduced streptococci could not have played an important role in the production of the endocarditis.

Heretofore this method has yielded sporadic and unreliable results at best except in a few isolated instances. Furthermore, although good results have been obtained in rabbits, only a few workers have reported positive results in dogs.

The question therefore arises as to why we were able to obtain such a large incidence of endocarditis in dogs with the simple intravenous injection. One possible explanation lies in the fact that we used streptococci isolated from another dog, while previous investigators worked with bacteria cultured from human sources. As stated, several workers have maintained that a certain degree of immunity in preventing extensive infection favors the localization of the inflammatory process on the valve. It is possible that in our experiments the dogs had some prior (natural or acquired) immunity to this organism, which, after all, was isolated from a dog. Such immunity, however, was obviously lacking in the dogs which showed no endocarditis.

Dietrich¹⁶ suggested that endocarditis produced by the simple intravenous injection is due to "activation" of the valve by repeated previous injections of living organisms over a long period of time. This explanation is not substantiated by our studies in view of the production of endocarditis by a single injection in 4 dogs. However, in the other 6 dogs, succumbing within from six to twelve days after the first of multiple injections, the establishment of such activation might have been possible. It is also unlikely that a preliminary impairment of the reticulo-endothelial function of the valve occurred, in view of the presence of endocarditis after a single injection. Furthermore our results cannot be explained according to the theory of elective localization, propounded by Rosenow,⁸ inasmuch as the source of the organism was the lung of a dog and not the cardiac valve.

An important point to be considered is the application of these results to the development of bacterial endocarditis in man.

Undoubtedly, the mechanical treatment, particularly the technic of preliminary injury, gives reliable results in the experimental animal. However, this procedure is not analogous to the development of endocarditis on healthy endothelium-covered valves, as often occurs in man. Although the introduction of a focus of inflammation by the method of Friedman and his co-workers¹³ also frequently led to endocarditis in animals, this procedure does not simulate the genesis of human endocarditis.

As stated, the hypothesis has been advanced in the last decade that there must exist a predisposition in the animal organism prior to the

development of experimental endocarditis. The actual production of endocarditis after preparatory treatment of the animal has been accomplished fairly constantly by some workers. Yet the data of the various investigators reveal that this method is not wholly dependable.

Our results, on the other hand, were secured without any preparatory injections. In fact, 4 of our animals acquired endocarditis after a single bacterial injection. The possibility of the existence of a natural predisposition to the development of endocarditis, of course, cannot be eliminated. Our work shows, however, that the artificial creation of a predisposing condition is not essential and that with organisms of sufficient virulence in the blood stream of animals of proper susceptibility endocarditis will develop even in the absence of any type of preliminary stimulating treatment.

The fact that bacterial endocarditis can be produced by so many methods with such a varied rationale suggests that a large number of factors may be involved in the development of this disease. Our experiments, however, clearly indicate that the presence of none of these several factors is essential and that virulent organisms per se in a previously healthy subject may be the sole cause of the development of acute bacterial endocarditis.

SUMMARY

Endocarditis was produced in 40 per cent of 25 dogs by simply injecting intravenously hemolytic streptococci isolated from a dog. In 4 dogs endocarditis was produced with a single injection. It is suggested that the use of a virulent strain isolated from the same species contributed to the positive results. It is concluded that numerous factors may be involved in the development of endocarditis. However, the presence of these several factors is not essential, for virulent organisms per se may cause acute bacterial endocarditis in a previously healthy animal.

PATHOLOGIC CHANGES IN RATS AND IN DOGS FED DIETS CONTAINING LEAD AND ARSENIC COMPOUNDS

COMPOUNDS USED: LEAD ARSENATE, ARSENIC TRIOXIDE, CALCIUM
ARSENATE AND LEAD ACETATE

LUCY L. FINNER, M.D.
AND
HERBERT O. CALVERY, Ph.D.
WASHINGTON, D. C.

This report contains the results obtained in an examination of four groups of rats and one group of dogs.

Each group of rats was comprised of four pairs. The members of each pair were litter mates, one of which was used as the experimental animal and the other as the control. The control in each pair was placed on diet A,¹ a diet relatively high in protein and composed primarily of natural food products, i. e., essentially of whole cereal grains, leguminous seeds and milk solids, so fortified in all accessory food factors that it met the requirements of the rat for growth and reproduction. The experimental member of each pair was also fed diet A, but with lead or arsenic added. The paired feeding method was followed.² The animals were placed on their respective diets at the age of 25 days. Since they were members of a larger group of animals, most of which were used for other investigations, the complete details of their history and treatment are presented elsewhere.

Group 1. The experimental rats in this group were placed on diet A, with the addition of 2.56 Gm. of lead as lead acetate per kilogram of diet, and were allowed to consume 500 Gm. of food, which required from six to seven weeks.

Group 2. The experimental animals of this group were placed on diet A, to which was added lead arsenate in such amounts that the lead content was 3.53 mg. per kilogram of diet. These rats were allowed to consume 1,000 Gm. of diet, which required from ten to twelve weeks.

From the Division of Pharmacology, Food and Drug Administration, United States Department of Agriculture.

Dr. G. E. Farrar was in charge of the pathologic investigations during the early stages of this study. Mr. C. E. Montgomery has been the technical assistant throughout the investigation. Dr. Esmond R. Long supplied the photomicrographs.

The investigation reported here was brought to a close long before it was complete by Congress when, on June 24, 1937, it passed the Agricultural Appropriation Act, containing the clause: "Provided further, that no part of the funds appropriated by this Act shall be used for laboratory investigations to determine the possibly harmful effect on human beings of spray insecticides on fruits and vegetables."

1. Laug, E. P., and Morris, H. J.: *J. Pharmacol. & Exper. Therap.*, to be published.

2. Mitchell, H. H., and Beadles, J. R.: *J. Nutrition* 2:225, 1930.

Group 3. The experimental diet in this group was diet A plus the equivalent of 215 mg. of arsenic per kilogram, added as the trioxide. The average time required to consume 500 Gm. was about six weeks.

Group 4. In this group the experimental diet was diet A with 215 mg. of arsenic per kilogram added as calcium arsenate. The time required by this group of animals to consume 500 Gm. of diet ranged from six to eight weeks.

Group 5. This group was composed of dogs. The report represents all the data we have had an opportunity to collect from a series of 29 dogs.

The history of the animals in the various groups and the data on their nutritional, chemical and therapeutic status will be published elsewhere: Group 1 and 2 (Laug and Morris¹); groups 3 and 4 (Morris and Wallace³); group 5 (Calvery, Laug and Morris⁴).

Although this report is not as complete as we should like, several important observations have been made, some of which are confirmatory of data already reported in the literature, yet all of which may furnish information for others who are interested in the same problem. The fundamental histologic changes following continued ingestion of small quantities of lead and arsenic over long periods are almost entirely unknown. Where in this paper negative results are reported, it is well to keep in mind that more extensive studies or special technics might have revealed changes not demonstrated in this study.

GENERAL METHODS OF EXAMINATION

Except when otherwise stated, the blood was examined just before the termination of the experiment. Red and white cell counts were made by the usual technic, and hemoglobin was determined by the use of the Newcomber disk in a Klett colorimeter. Differential white cell counts were not made. Smears stained by Wright's or Giemsa's technic were carefully examined qualitatively for morphologic changes in the blood. In the blood of lead-treated animals and their controls a special search was made for basophilic stippling of red cells. Since in many of the experimental and control animals reticulocytes were relatively high,⁵ comparative studies were not attempted.

The animals were weighed and then killed by decapitation. Qualitative observations were made of the carcass externally, and of the viscera, skeleton, brain and cord.

Blocks of the heart, lungs, liver, spleen, stomach, duodenum, ileum, large bowel, pancreas, testes or ovaries, kidneys and adrenals were hardened in Zenker's solution (without acetic acid) or in solution of formaldehyde U. S. P. diluted 1:10. The brain and cord were fixed in the latter solution. Embedding was done in paraffin, and sections for microscopic examination were stained with hematoxylin and eosin.

3. Morris, H. J., and Wallace, E. W.: *J. Pharmacol. & Exper. Therap.*, to be published.

4. Calvery, H. O.; Laug, E. P., and Morris, H. J.: *J. Pharmacol. & Exper. Therap.*, to be published.

5. There is some evidence that at least a part of the rat colony was infected with *Bartonella*. Such an infection would explain the high reticulocyte count. Under normal conditions a high reticulocyte count is the only sign of a *Bartonella* infection.

The bone marrow was examined by the technic described by Farrar,⁶ which consists in enumeration of the nucleated cells after quantitative dilution of the marrow with 1 per cent acetic acid. When properly carried out, this procedure seems to afford an accurate evaluation of the degree of marrow activity.

SPECIAL TECHNIC FOR EXAMINATION OF LEAD-TREATED ANIMALS

Preparations of the skeletal tissues were made according to a modification of the method reported by Sieber,⁷ which consists essentially in the following: The bones are fixed from one to three weeks in solution of formaldehyde U. S. P. diluted 1:10 and saturated with hydrogen sulfide. When lead is present in large quantities, it becomes visible as black lead sulfide within a few minutes after treatment with formic acid. No lead sulfide is visible before addition of the acid. In a few cases, sections of the bone treated as described and impregnated with paraffin were prepared for microscopic examination.

Similar attempts were made to demonstrate lead in the soft tissues. With this end in view, blocks of the kidneys, spleen, liver, pancreas, intestine and brain were fixed for two weeks or longer in solution of formaldehyde U. S. P. (1:10) which had been saturated with hydrogen sulfide. They were then embedded in paraffin by the ordinary methods and cut into sections for microscopic examination. Where lead was present, it was seen as black deposits of lead sulfide, the visibility and localization of which were improved by counterstaining with 0.5 per cent saffranin in 50 per cent alcohol.

No special technics were applied to tissues of the arsenic-treated animals.

OBSERVATIONS

GROUP 1.—The experimental rats in this group consumed, over a period of from six to seven weeks, 500 Gm. of food containing 2.56 Gm. of lead as lead acetate per kilogram of diet. External examination revealed general impairment of nutrition and dulness of the hair. The mouths and teeth were clean and appeared normal, with no lead lines.

The hemoglobin was low (averaging 17 per cent less than that of the controls), and the red and white cell counts were slightly decreased (6.6 and 5.6 per cent, respectively). Morphologic examination of the blood revealed basophilic stippling of the red cells in specimens from all of the lead-treated rats, while this was not observed in the controls.

Autopsies revealed no gross changes in the viscera. There were, however, weight differences between some of the viscera of the experimental animals and those of the controls. The kidneys, spleens and hearts of the lead-treated animals as compared with the controls showed average weight increases of 22, 20 and 10 per cent, respectively, while the testes averaged 11 per cent less. These weight changes were consistent, being of about the same magnitude in each animal.

The kidneys were smooth and of normal color, the brains and cords appeared grossly normal, the bone marrow of the femurs was hyperplastic, being more red than that of the controls, and the skeletons

6. Farrar, G. E.: *Am. J. Physiol.* **117**:662, 1936.

7. Sieber, E.: *Arch. f. exper. Path. u. Pharmacol.* **181**:273, 1936.

in the fresh state revealed no abnormalities except that the bones of the experimental animals were very fragile. The intestines also appeared grossly normal except that a few centimeters above the ileocecal valve and extending throughout the cecum and large intestine the contents were black, due, undoubtedly, to the formation of lead sulfide.

A femur from each rat was preserved and decalcified in the presence of hydrogen sulfide as described in the section on pathologic methods. The results obtained were striking in that the entire femur turned black from conversion of the lead deposits into lead sulfide. One femur appeared spotted, black flakes appearing on the bony background, and under low magnification it was apparent that the black flakes represented deposits of lead sulfide about the haversian canals.

On longitudinal section of each completely darkened femur, the lead sulfide was seen to penetrate throughout the bony substance of the shaft, the marrow remaining free from discoloration. No lead line was present at the epiphysial junction.

One entire rat skeleton was treated according to the technic described. All the bones disclosed heavy deposits of lead sulfide, the heaviest being observed in the long bones, where deposits were more dense in the shafts than at the ends.

The cartilages of the thorax and those of the larynx and trachea also revealed heavy deposits of lead sulfide. However, smaller amounts occurred in the cartilages than in the bones.

Microscopic examination of the viscera gave negative results except as regards the kidneys. The changes in the kidneys were marked and were quite uniform in all specimens. The epithelial cells lining the tubules were very irregular in size. Some of the nuclei were tremendously hypertrophied, and in many of the nuclei eosin-staining bodies were present, similar to the nuclear inclusion bodies described by Blackman⁸ in the kidneys of persons dying of lead poisoning. In some of the tubules these abnormal epithelial cells had sloughed into the tubular lumens. Occasionally several such sloughed cells had clumped together, forming a cast. The tubular changes were most marked in the medulla but were also present to a lesser extent in the cortex. They appeared essentially alike whether the material was fixed in solution of formaldehyde or in Zenker's solution. The glomeruli appeared uninjured, and the blood vessels were not sclerotic. No scars or inflammatory reactions were present.

Sections of kidney tissue which had been treated with hydrogen sulfide and counterstained with saffranin revealed particles of lead sulfide occupying some of the nuclei of the tubular epithelium. In a few cases, fine particles of lead sulfide occurred also in the cytoplasm. The lead

8. Blackman, S. S., Jr.: *Bull. Johns Hopkins Hosp.* 58:384, 1936.

sulfide bodies within the nuclei resembled in size and position the eosinophilic inclusion bodies which were seen in sections stained with hematoxylin and eosin.

Throughout the medulla and to a lesser extent in the cortex there were large clumps of lead sulfide. Some of the clumps were round and somewhat larger in diameter than the renal tubule. Other clumps had an irregular shape. Some of the lead sulfide masses in the medulla were elongated and followed the directions of the renal tubules. It is probable that they represented casts of distended tubules around which the tubular structure had been destroyed.

Sections of the kidneys from the control animals were entirely free from all the tubular changes described for the experimental animals.

It is probable that the increase in weight of the kidneys of the lead-treated animals was caused by the modifications of the tubular epithelium.

Sections of the spleens which were examined by the prussian blue reaction revealed heavy deposits of iron in the lead-treated animals, while very little iron was demonstrable in the spleens of the controls.

Microscopic examination of cross sections and longitudinal sections of the femur revealed countless particles of lead sulfide. They were arranged, for the most part, in a radiating pattern about the haversian canals. Many particles occurred also in a zone beneath the periosteum, whereas none could be seen in the marrow. The black lead sulfide particles in the bone were easily distinguished under the high-dry and oil immersion objectives. Very small and sparsely distributed particles were most easily demonstrated by use of the dark field with an oil immersion objective. Sections of other organs and tissues revealed no definite changes.

GROUP 2.—The experimental rats in this group consumed, over a period of from ten to twelve weeks, 1,000 Gm. of food containing 3.53 mg. of lead as lead arsenate per kilogram of diet. All observations, both macroscopic and microscopic, were essentially normal.

GROUP 3.—Over a period of six weeks the experimental rats in this group consumed 500 Gm. of food containing 215 mg. of arsenic as the trioxide per kilogram of diet. The general nutritional state of all the animals was good. Autopsy revealed gross changes in the intestinal tracts of the arsenic-treated rats. While the small intestines of all the controls contained abundant food, those of the arsenic-treated animals contained only a colorless mucus. The cecums of all animals which had ingested arsenic trioxide were markedly distended and contained putty-colored mushy fecal material, while the large intestines appeared normal.

The nucleated cell counts of the bone marrow, averaging 2,556,000 for the experimental animals, compared with 2,284,000 for the controls, suggested slight hyperplasia of the bone marrow.⁶ The skeleton

revealed no abnormalities, and the brain and cord appeared normal. Microscopic examination of the viscera revealed capillary dilatation in the lungs, kidneys, adrenals and intestines. The kidneys otherwise appeared normal.

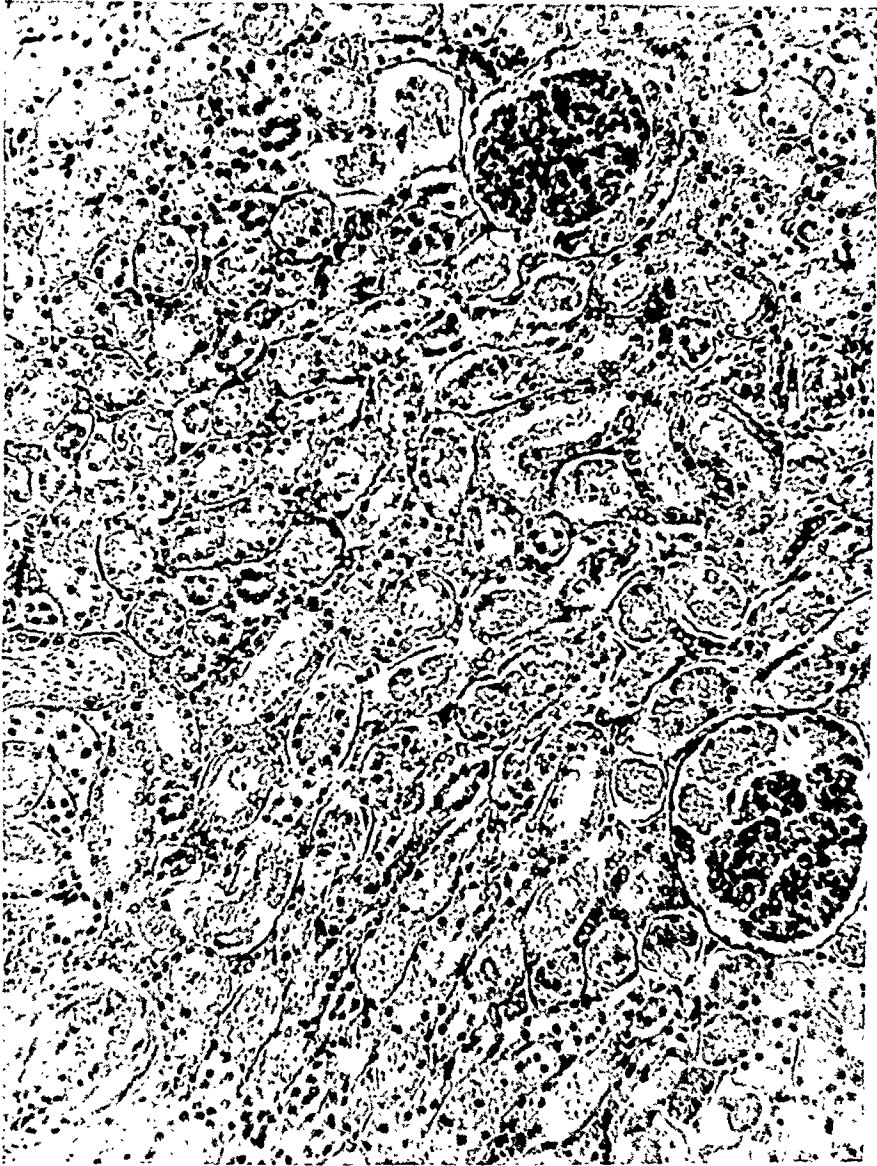


Fig. 1 (dog 1).—The photomicrograph shows protein in the tubular lumens and in Bowman's space in the glomeruli. In addition, tubular epithelium is found in Bowman's space. This is not entirely an artefact. It represents desquamation of the tubular epithelium at the outlet of the glomerular space, occurring as a result of injury. The subsequent forcing back into Bowman's capsular space is presumably an artefact induced in the postmortem examination.

GROUP 4.—The experimental rats in this group consumed 500 Gm. of food containing 215 mg. of arsenic as calcium arsenate per kilogram

of diet, over a period of from six to eight weeks. The animals receiving calcium arsenate were slightly larger than the controls. The teeth and mouths were in good condition, the paws and skin appeared normal, and the coats were glossy. The blood picture was essentially normal.

Postmortem examinations disclosed more fat in the abdominal walls and about the viscera in the treated animals than in the controls. There was edema of the abdominal subcutaneous tissue, peritoneum and intra-abdominal fatty tissue. The vessels supplying the intestines, uterus and testes were markedly dilated. The lungs were abnormally red from dilatation of the capillaries. The liver was enlarged, lustrous and grayish red, and had a watery appearance. The small intestines were extremely edematous, the edema being most pronounced near the ileocecal junction. The length of the small intestines was significantly increased in all of the arsenic-treated animals, and the cecum was markedly distended.

Nothing abnormal was seen in the stomach and large intestines. The mucosa of the small intestines was edematous, but there were no hemorrhages. The cecum in each of the poisoned rats contained large quantities of mushy yellow fecal material.

In the arsenic-treated females, the uterine horns were elongated, and the entire organ was flabby. No abnormalities were noted in the heart or pancreas. The spleen and kidneys revealed no change except that they weighed 18 per cent and 31.4 per cent, respectively, more in the arsenic-treated rats than in the controls. The brain, the spinal cord, the skeleton and the bone marrow of the femur appeared grossly normal in all cases. The edema in this group of animals was confirmed in the larger number used for chemical investigations,³ and this accounts for the increased weight of the experimental animals as compared with the controls since the dry weights were the same.

Microscopic examinations were not made.

GROUP 5.—The histories of only a few dogs, which are representative of the whole group, will be given in detail. For a more complete presentation of the histories, symptoms, nutritional status and chemical findings, see Calvery, Laug and Morris.⁴

Dog 18.—This was a young animal, 46 days of age, when placed on diet B¹, a modified Cowgill diet, containing 64 mg. of lead as lead acetate per kilogram. The average lead intake per kilogram per day was 1.5 mg. On the forty-third day of the experiment, at 8 o'clock the dog showed the first severe signs of intoxication. When first observed, it began barking and failed to respond to the usual attempts to quiet it. When the cage was opened, it leaped out and ran wildly around the room, trying to find a place to hide. Shortly afterward mild attacks of convulsions occurred, which grew worse and occurred more frequently until the final severe attack at 1 p. m., when it died.

The autopsy was performed shortly after death. The carcass was that of a well nourished dog in opisthothonos with marked rigor mortis. There was foaming at the mouth. The skin was in good condition. The teeth were dirty, but the oral cavity appeared otherwise normal. The thoracic and abdominal viscera, including the kidneys, appeared grossly normal. The urinary bladder was empty. The brain was moderately edematous and hyperemic. Smears of the brain examined

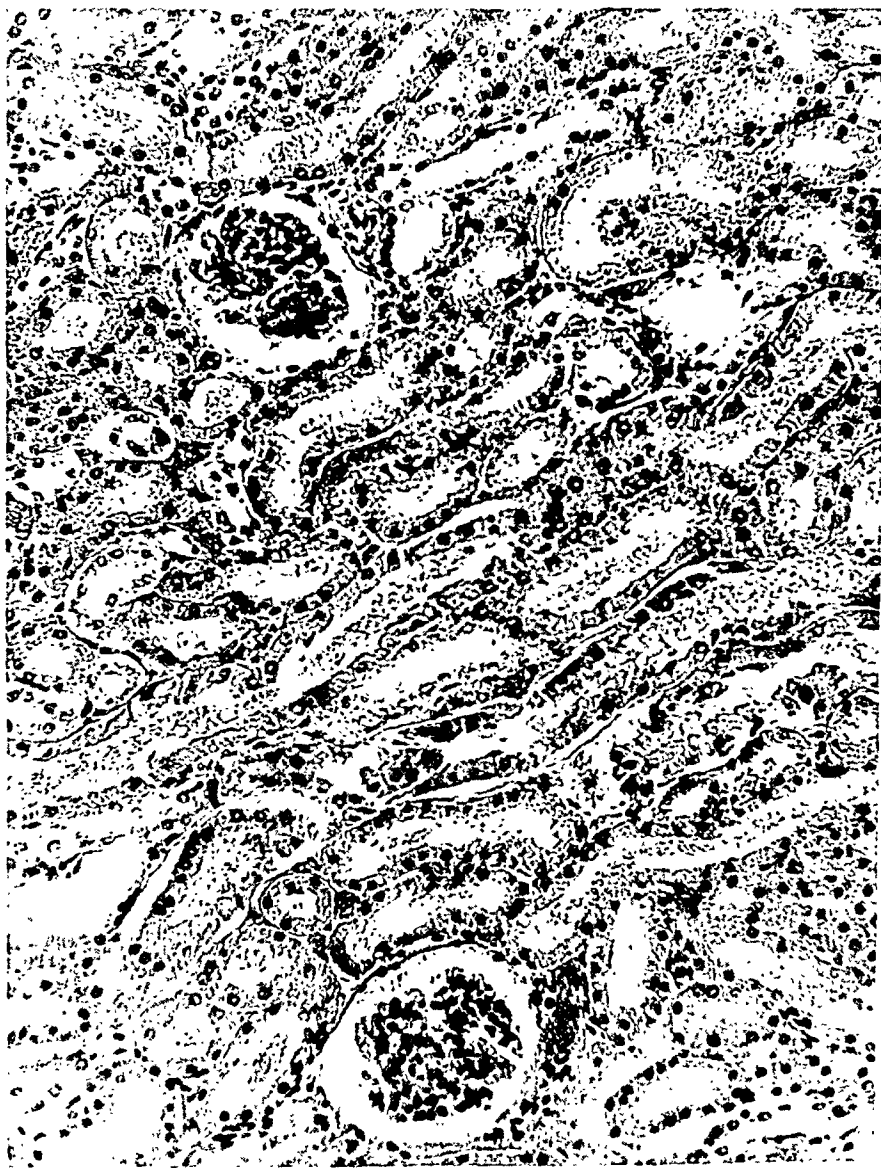


Fig. 2 (dog 10).—The photomicrograph shows tubular degeneration with epithelial desquamation and marked proteinuria; $\times 240$.

for Negri bodies revealed none. Postmortem blood smears stained by Wright's method revealed basophilic stippling in a few red cells. The skeleton in the fresh state appeared entirely normal. The marrow of the femur was abnormally red. Bones treated with hydrogen sulfide in solution of formaldehyde U. S. P. and decalcified in formic acid turned brown as a result of the formation of lead sulfide.

Microscopically, sections of the heart muscles appeared normal. In the lungs, liver and spleen moderate congestion was noted. The stomach, small intestine and colon disclosed nothing abnormal. No changes were seen in the pancreas or adrenal.

Sections of kidneys revealed nothing abnormal in the capsule, glomeruli or blood vessels. However, marked changes were present in the tubules. The

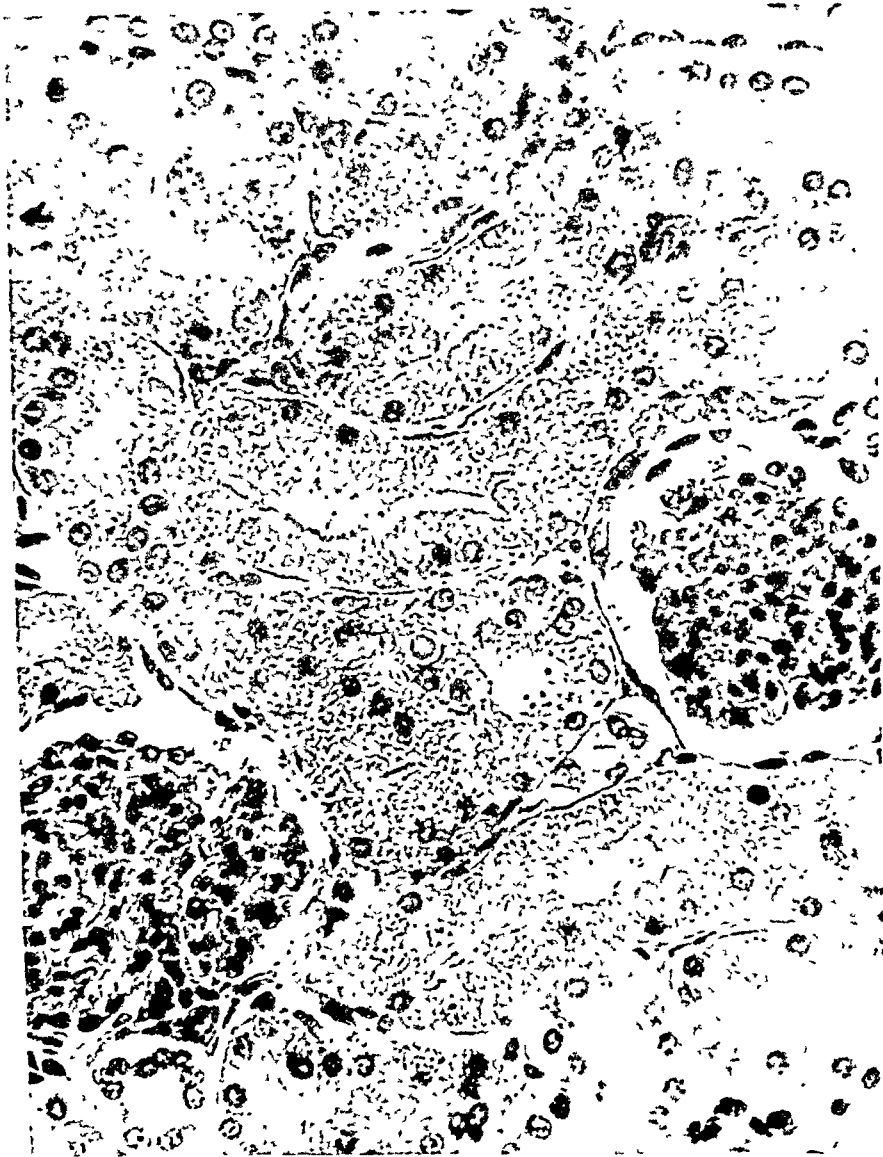


Fig. 3 (dog 18).—The photomicrograph shows mild tubular degeneration, with isolated cell injury; $\times 720$. A striking feature is the presence of sharply defined spherical bodies, lying either in or adjacent to cellular cytoplasm, with about the size of the nucleus of a tubular epithelial cell. One such body is seen close to the center of the section (such bodies have been seen in human kidneys after long continued administration of lead and bismuth).

tubular epithelial cells were irregular in size, and many contained hypertrophied nuclei. Many injured epithelial cells had sloughed into the lumen. The changes noted in the epithelial nuclei were very different from those observed by us in

lead-poisoned rats and from those described by Blackman⁸ in the kidneys of children who had died of lead poisoning, in both of which the characteristic change consisted in the presence of acidophilic inclusion bodies in the nuclei. No such bodies were noted in the kidneys of this dog. On the other hand, the damaged nuclei were broken into many fragments, which were scattered throughout the cell. The picture resembled what one would imagine to occur if an intranuclear explosion had taken place. A great many of the tubular epithelial cells were damaged in this way. In addition to the nuclear changes, inclusion bodies occurred in the cytoplasm. These closely resembled the bodies found by Pappenheimer and Maechling⁹ after administration of bismuth. The picture was the same whether the material was fixed in solution of formaldehyde or in Zenker's solution.

Sections of kidney which had been treated with hydrogen sulfide while being fixed in solution of formaldehyde revealed small black particles in the capillary endothelium and in the basement membrane of the renal tubules. No lead sulfide particles were noted in the tubular epithelial cells. In this respect, again, the dog's kidney differed from that of the rats, in which lead sulfide particles were found within the tubular epithelial nuclei.

Sections of the brain and cord stained with hematoxylin and eosin revealed no cellular changes. The vessels appeared normal. Special staining for lead did not reveal its presence in the brain or cord.

A cross section through the shaft of the femur, which had been treated with hydrogen sulfide, showed particles of lead sulfide deposited around the haversian canals. A longitudinal section through the upper end of the femur revealed heavy deposits of lead at the junction of the diaphysis and epiphysis.

In a section of striated muscle taken from the quadriceps femoris there were small patches of lymphocytic infiltration between the muscle fibers. At the beginning of this experiment the red blood cell count was 6,375,000, and two weeks before death the count was 4,740,000.

Dog 8.—This dog was 123 days of age when placed on the same diet as dog 18. After thirty-one days on this diet, the animal went into severe convulsions, from which it recovered after being given 1 Gm. of magnesium sulfate intramuscularly followed by diet B with the addition of calcium. After the dog had been on this diet three days, it was placed on diet B for thirty-five days. After the total interim period of thirty-eight days, it was put back on the experimental diet. On the ninety-first day following the return to the experimental diet, it showed jerky muscular movements on the right side. It was also apathetic and passed urine which was bright red with blood. It was found dead in its cage the following morning.

The examinations of the blood of dog 8 are recorded in table 1.

The carcass was that of a well nourished, well developed dog. No rigor was present, and the carcass was no longer warm.

The thoracic and abdominal viscera appeared normal with the exception of the small intestines, spleen and kidneys. The intestinal mucosa was slightly hemorrhagic. The spleen was enlarged and firm. The kidney had a bluish gray appearance. The capsules appeared thickened but stripped easily, leaving smooth surfaces. The bladder contained bloody urine although the mucosa was intact.

The bones were hard and brittle. The shaft of the femur was markedly thickened, leaving only a small cavity for marrow. The marrow was small in

9. Pappenheimer, A. M., and Maechling, E. H.: *Am. J. Path.* **10**:577, 1934.

amount and grayish red. The skull was much thickened and coarsely porous. The large pores in the skull were filled with very red marrow.

The brain was extremely edematous, but the meninges appeared grossly normal.

Only the kidneys were examined microscopically, and these presented a picture like that described in the kidneys of dog 18; i. e., there was degeneration of the tubular epithelium with fragmentation of the epithelial nuclei.

Dog 7.—This dog was 141 days of age when started on diet B containing 12.8 mg. of lead as lead acetate per kilogram of diet and was on the diet for one hundred and forty days. The average intake of lead per kilogram of body weight per day was only 0.33 mg. The animal was found dead on the morning of April 1, 1937. There had been no signs of previous illness except that on the one hundred and nineteenth day it refused food for one day and on the day before death showed some apathy and no desire for food. The blood of this dog was not studied.

At autopsy the brain was hyperemic but not edematous. The teeth were dirty. The duodenal and jejunal mucosa was slightly hemorrhagic. There was bloody urine in the bladder although the mucosa was intact. The kidneys and the remaining viscera showed nothing grossly abnormal. The skeleton showed nothing of interest.

TABLE 1.—*Examinations of Blood of Dog 8*

Date	Hemo- globin, Gm. per 100 Cc.	Red Blood Cells per Cu. Min.	White Blood Cells per Cu. Min.	Results of Examination of Smear
12/12/36*	10	5,150,000	37,000	Many stippled cells; many nucleated cells, most of which showed basophilic stippling of the cytoplasm; moderate polychromasia; moderate anisocytosis
12/14 36	12.7	5,250,000	14,000	Many stippled red corpuscles; a few nucleated red cells; more polychromasia than in preceding smear
1/ 8 37	12.2	5,950,000	17,950	Many stippled red cells; no nucleated red cells; polychromasia as on Dec. 14.

* The dog had a convulsion on this date and was taken off the lead diet.

Only the kidneys were examined microscopically. They showed tubular degeneration similar to that described in the kidneys of dogs 8 and 18.

Dog 6.—This dog was 141 days of age when placed on the same level of lead intake (0.33 mg. per kilogram of body weight) as dog 7 and was found dead on the morning of April 27, 1937, after it had been on the diet one hundred and sixty-seven days. There had been no apparent illness except a refusal of food on the one hundred and fifty-third day and a show of some apathy as well on the day before death.

At autopsy the teeth were covered with a dirty brown deposit. There was a foul odor to the mouth.

The mucosa of the small intestine was hemorrhagic. The heart, lungs, liver and pancreas revealed nothing of interest. The spleen was slightly enlarged and firm. The kidneys were bluish gray. The capsule was slightly adherent, but the renal surface beneath the capsule was smooth. The bladder contained bloody urine but the mucosa was intact. The brain was hyperemic but not edematous.

The shaft of the femur was increased in thickness, and the bone was very hard. The marrow cavity of the femur was reduced. The marrow was small in amount and abnormally red. There was compensatory formation of marrow in the skull, similar to that described in dog 8 but somewhat less marked.

Microscopic examinations were made only of the kidney, and the observations were essentially the same as those on the kidneys of dogs 7, 8 and 18.

Dog 17.—This dog was a litter mate of dog 18 and was placed on the same diet at the same time. The first symptoms appeared at the fourteenth day as weakness of the front legs and mild paralysis of the right hindleg. The paralysis grew steadily worse until the thirtieth day, when both hindlegs were completely paralyzed, and the animal refused to eat. At that time it was taken off the diet containing lead and placed on a high calcium diet. The dog never recovered completely from the paralysis, although it greatly improved. After an interim period of two hundred and fourteen days, it was again placed on the lead-containing diet. During the second period of ingestion of lead the animal was bred and rapidly gained weight while showing the usual signs of pregnancy. At the end of the length of time of the usual gestation period, when the abdominal enlargement had disappeared and no pups were born, the dog was killed for further observation at autopsy. The blood picture is presented in table 2.

The carcass was that of a well nourished female dog. The skin was in good condition. There was atrophy of the thighs. The mouth was clean, and the teeth were all present, but some of the teeth contained small black blemishes which could not be removed by scraping.

TABLE 2.—*Examinations of Blood of Dog 17*

Date	Hemo- globin, Gm. per 100 Cc.	Red Blood Cells per Cu. Mm.	White Blood Cells per Cu. Mm.	Results of Examination of Smear
6/26/36	Not done	5,650,000	26,000	Negative
7/ 8/36	Not done	4,265,000	27,400	Negative
8/ 5/36	12.0	3,990,000	14,150	A few stippled red corpuscles (dog taken off the diet containing lead)
8/10/36	12.3	4,735,000	12,400	Many stippled red cells
1/ 8/37	13.2	6,250,000	11,300	No stippled red cells seen (dog returned to diet containing lead)
4/29/37	14.6	6,700,000	11,500	Moderate number of nucleated red cells; moderate number of stippled red cells

Gross examination of the thoracic and abdominal viscera exhibited nothing noteworthy with the exception of the uterus. *Both horns of the uterus contained the remnants of resorbing fetuses.* The brain and the cord appeared grossly normal. The skeleton in the fresh state revealed nothing of interest.

Microscopic examination of the kidney revealed only moderate tubular changes.

Controls.—The autopsies on the control animals all disclosed essentially normal conditions.

COMMENT

Pathologic examinations have been made of groups of rats receiving diets containing different compounds of lead and arsenic. The animals in each case were taken from larger groups, the other members of which were used for chemical investigations, which are reported separately.⁴

The rats of group 1, receiving a diet containing 2.65 mg. of lead per gram until they had consumed 500 Gm. of diet, showed dulness of hair, moderate secondary anemia associated with basophilic stippling of the red cells, large deposits of iron in the spleen and a hyperplastic bone marrow. By a special method of treatment enormous quantities of lead were found in the bones and cartilages; large quantities were

also present in the kidneys, but none was demonstrated in other organs. The long bones were increased in thickness and were abnormally hard and brittle. This observation was extensively confirmed in the animals used for chemical investigations.¹

The kidneys showed marked irregularity of the tubular epithelium, with hypertrophy of the nuclei of many of the tubular epithelial cells. Many of these nuclei contained eosinophilic inclusion bodies similar to those described by Blackman⁵ in the kidneys in cases of lead poisoning in man. Inclusion bodies also occurred in the cytoplasm. These closely resembled the bodies found by Pappenheimer and Maechling² after administration of bismuth. Special treatment of the kidneys with hydrogen sulfide revealed lead inclusion bodies in the nuclei, which were similar in size and position to the acidophilic bodies mentioned. It is believed that these two types of inclusion bodies may have the same origin.

Microscopic examinations made of the animals in group 2, receiving very small quantities of lead arsenate, showed no abnormalities of definite significance.

Observations made on the animals of group 3, which had received 500 Gm. of diet containing 107.5 mg. of arsenic as arsenic trioxide, revealed a more or less generalized dilatation of capillaries in the viscera and parenchymatous degeneration of the liver. There were changes in the small intestine and distention of the cecum. The bone marrow was slightly hyperplastic.

The animals of group 4, receiving calcium arsenate, were significantly heavier than the controls, and autopsies showed large deposits of subcutaneous and abdominal fat. However, the increase in weight was entirely due to edema, since the average dry weight of a large number of animals from this same group used for chemical examination was almost the same as that of the controls.³ There was marked edema of the subcutaneous and intra-abdominal fatty tissues and apparently also of the liver. The small intestine and the uterine horns were elongated, and the cecum was distended, which was probably due to muscular relaxation. There was generalized capillary dilatation, but the bone marrow and blood pictures were not markedly different from those of the controls. It is interesting that this group of animals showed much more marked signs of intoxication than did those of group 3 receiving arsenic at the same level. This is contrary to the usual conception that arsenic is more toxic in the trivalent form than in the pentavalent form.

Although a total of 29 dogs were used and observed post mortem, the protocols of only a few of them are presented. Of the 20 dogs receiving lead in their diet, 15 died before the termination of the experiment. All of the signs, symptoms and data from examinations indicated that no factor other than the lead included in their diet, even the very low level of 0.33 mg. of lead per kilogram of body weight per day,⁴

was involved in causing their death. Not 1 of the 7 dogs receiving diets to which no lead was added showed any signs of abnormality up to the time when they were put to death, at the termination of the experiment. Of the latter group, some were kept for over a year and had been raised from pups on the basic diet, diet B.

The kidneys of all the dogs which had been on diets containing lead up to the time of death showed tubular degeneration. The type of degeneration was different from that seen in the rats. In 3 dogs the bladder contained bloody urine, though the mucosa was intact. In all dogs which had had convulsions, hyperemia and edema of the brain were noted. The bones of many of the dogs receiving diets containing lead were hard, brittle and thickened, and in the long bones of this description the marrow cavities were constricted. Compensatory formation of marrow was noted in the skull. The lead deposits in the bones of the dogs resembled those seen in the rats. The dogs on lead whose blood was examined all had stippled red cells. One dog had nucleated red cells with basophilic stippling of the cytoplasm.

The pathologic investigations taken in conjunction with the extensive chemical investigations⁴ indicate clearly that the ingestion of lead even at very low levels results in severe injury to the tissues of animals and ultimately in their death. Dogs 6 and 7, reported on here, received only 0.33 mg. of lead as lead acetate per kilogram of body weight per day. One of them died on the one hundred and fortieth day and the other on the one hundred and sixty-seventh day of the regimen. Five animals in this series received lead arsenate, and the results were the same as in the animals receiving lead acetate at the same level of lead in the diet. To make more certain of this, litter mates were used in some cases. The most striking differences were those due to age, younger animals being much more susceptible than older animals receiving the same level of lead.

SUMMARY

The microscopic observations reported in this paper bear out the chemical results reported elsewhere and show that under the conditions of our experiments injuries of tissues were caused by both lead and arsenic. The most marked histologic changes were produced in the kidney. The most severe injury and death resulted from the feeding of lead. Some dogs fed as little as 0.33 mg. of lead per kilogram of body weight per day died before the scheduled termination of the experiment. The manner of death of some of these animals corresponded in almost every particular with the convulsive terminations in cases of human lead poisoning described in the literature. In view of these results the conclusion is inescapable that lead and arsenic are chronic tissue poisons, the continued intake of which into the body is always fraught with the possible production of definite injury.

PATHOLOGIC EFFECTS OF CERTAIN GLYCOLS AND RELATED COMPOUNDS

H. D. KESTEN, M.D.

M. G. MULINOS, M.D.

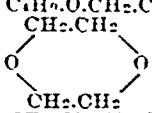
AND

LEO POMERANTZ, B.S.

NEW YORK

A number of polyhydric alcohols and their derivatives are being used in increasing quantities in many industries. They are used, for example, in the manufacture of lacquers, flavoring extracts, textiles, cosmetics, pharmaceuticals, preservatives, detergents and polishes. As part of a study of the toxicology of ethylene glycol (glycol), diethylene glycol (diglycol), propylene glycol and some of their derivatives, the organs of rats, rabbits and guinea pigs to which these substances had been administered were studied morphologically. The degenerative changes that occurred in some of these organs form the subject of this report.

TABLE 1.—*Chemical Formulas of Compounds Used**

Compound	Structural Formula
Ethylene glycol; glycol.. . . .	$\text{HO}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$
Ethylene glycol diacetate	$\text{CH}_3\cdot\text{CO}\cdot\text{O}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{O}\cdot\text{CO}\cdot\text{CH}_3$
Propylene glycol	$\text{HO}\cdot\text{CH}_2\cdot\text{CH}\cdot\text{OH}\cdot\text{CH}_3$
Diethylene glycol; diglycol.. . . .	$\text{HO}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{O}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$
Ethyl diethylene glycol (diglycol monoethyl ether); carbitol	$\text{C}_2\text{H}_5\cdot\text{O}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{O}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$
Methyl diethylene glycol (diglycol monomethyl ether); methyl carbitol	$\text{CH}_3\cdot\text{O}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{O}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$
Butyl diethylene glycol (diglycol mono n butyl ether); butyl carbitol	$\text{C}_4\text{H}_9\cdot\text{O}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{O}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$
Diethylene dioxide (dioxane)	
Dipropylene glycol	$\text{CH}_2\cdot\text{OH}\cdot\text{CH}\cdot\text{CH}_2\cdot\text{O}\cdot\text{CH}_2\cdot\text{CH}\cdot\text{OH}\cdot\text{CH}_3$

* The compounds were of commercial grade and were manufactured by the Carbide and Carbon Chemicals Corporation.

The chemical relationship of the several glycols used is indicated in table 1.

The compounds were given by mouth to rats and guinea pigs, usually in the drinking water, and by mouth or intravenously to rabbits, for from one to two hundred and thirty-four days. The animals were fed stock laboratory diets, and they were maintained in a state of good nutrition except as noted. The tissues of animals that died during the experiments or that were killed were promptly

From the departments of pathology and pharmacology, College of Physicians and Surgeons, Columbia University.

The expenses of the research of which this investigation is a part were defrayed in part by the Philip Morris Fund.

fixed in Zenker's solution, cut in paraffin and stained with hematoxylin and eosin. (The tissues of many animals dying during the night were discarded because of postmortem changes; none of these animals are included in this report.) Certain blocks were also fixed in formaldehyde for the study of fat and in absolute alcohol for the study of glycogen by Best's method.

The water content of the liver and kidneys was determined for certain of the animals by drying to constant weight at 90 C. The nonprotein nitrogen of the whole blood was determined by the micro-Kjeldahl technic for most of the animals that were killed at the end of an experiment.

ETHYLENE GLYCOL

The simplest member of the group of substances used is ethylene glycol, or, as it is often called, glycol. The effects of glycol have been extensively studied, and have been reviewed, together with the literature, by Browning.¹ Pathologically, they consist of deposition of masses of calcium oxalate in the renal tubules, with oxaluria and in some cases hematuria.

Eleven rabbits received from 1 to 4 cc. per kilogram of body weight of glycol intravenously in a single dose. They were killed at the end of from one to one hundred and twenty days. Four of the 11 presented the lesions to be described (as early as twenty hours after a dose of 4 cc. per kilogram) with a rise in the nonprotein nitrogen of the blood to 226 mg. in 100 cc.² Thirty rats received glycol in from 1 to 5 per cent concentration in the drinking water for from eight to seventy-nine days.³ Thirteen died in from nine to fifty-one days, and 10 of them had lesions similar to those in the rabbits. Seventeen were killed at the expiration of from eight to seventy-nine days, and the kidneys of 10 were involved in the same way. Approximately 6 cc. of glycol per kilogram of body weight is required daily for nine days to cause renal lesions and death.

The renal lesions are characterized by slight enlargement of the kidneys, associated with many gray glistening streaks and dots in the cortex and medulla. These streaks consist of deposits of refractive, colorless or pale greenish granular and crystalline material in the tubules, chiefly the convoluted. Only a few tubules may be involved, or the deposits may be widespread. In the latter case, dilatation of the tubules proximal to the crystalline deposits may occur. In and about the plugged tubules a mild acute inflammatory reaction is sometimes set up. In some instances, a few epithelial cells are destroyed, and regeneration sets in from those remaining. The crystalline masses reduce silver nitrate and are soluble in 10 per cent nitric or sulfuric acid. They are composed at least in part of calcium oxalate.

1. Browning, E.: Toxicity of Industrial Organic Solvents, Medical Research Council, Industrial Health Research Board, Report 80, London, His Majesty's Stationery Office, 1937.

2. The normal range of nonprotein nitrogen in the blood of control rats and rabbits is from 30 to 55 mg. in 100 cc.

3. Except when stated otherwise, the average daily intake of glycol water in these experiments was approximately 30 cc. per rat.

ETHYLENE GLYCOL DIACETATE

No previous study had been made of the effect on animals of the acetic acid ester of glycol.

Eleven rats received this chemical in from 1 to 5 per cent concentration in the drinking water for from seven to one hundred and thirty days. Four died in from seven to one hundred and fourteen days; lesions of the kidneys were present in all. The remaining 7 were killed at intervals of from fifteen to one hundred and thirty days; the kidneys of 4 were affected. The minimal dose required to produce damage in the kidneys was approximately 6 Gm. per kilogram daily, received in 5 per cent concentration, for seven days.

The kidneys of the animals affected contain deposits of calcium oxalate quite like those produced by glycol (fig. 1 *A*). Dilatation of the proximal portion of the convoluted tubules may also be present.

Further evidence of the composition of the crystalline deposits was obtained by analyzing the kidneys of 4 of the rats for calcium by the method of Halverson and Bergeim.⁴ An unaffected kidney contained 9.2 mg. of calcium in 100 Gm. wet weight of kidney, an amount that is within the normal range. Three kidneys in which the crystalline deposits were found histologically contained 86.2, 104.1 and 819.2 mg. of calcium in 100 Gm. wet weight, respectively. The deposits were sufficiently large in the last animal to cause dilatation of the convoluted tubules proximally and slight nitrogen retention (blood nonprotein nitrogen—76.2 mg. in 100 cc.). However, no enlargement or hyperplasia of the parathyroid glands of any of these rats was demonstrable when the glands were sectioned serially. This is of interest in view of the well established sequence of renal insufficiency, hyperparathyroidism and increase in renal calcium.⁵

PROPYLENE GLYCOL

Of the several substances investigated, propylene glycol was the most innocuous. This fact has been emphasized by other investigators.⁶ In vitro or when injected intravenously, it is hemolytic,^{6a} but taken into the alimentary tract it is readily absorbed and rapidly oxidized. Lehman and Newman^{6d} assigned to it a narcotic value of about one-third that of ethyl alcohol.

4. Halverson and Bergeim, cited in Peters, J. P., and Van Slyke, D. D.: *Quantitative Clinical Chemistry—Methods*, Baltimore, Williams & Wilkins Company, 1932, p. 767.

5. Donohue, W.; Spingarn, C., and Pappenheimer, A. M.: *J. Exper. Med.* **66**:697, 1937.

6. (a) Hunt, R.: *Indust. & Engin. Chem.* **24**:836, 1932. (b) Seidenfeld, M. A., and Hanzlik, P. J.: *J. Pharmacol. & Exper. Therap.* **44**:109, 1932. (c) Hanzlik, P. J.; Mehrtens, H. G., and Spaulding, J. B.: *ibid.* **49**:300, 1933. (d) Lehman, A. J., and Newman, H. W.: *ibid.* **60**:312, 1937. (e) Holck, H. G. O.: *J. A. M. A.* **109**:1517, 1937.

EXPLANATION OF FIGURE 1

A, calcium oxalate crystals in convoluted tubules of the kidney of a rat which received ethylene glycol diacetate in a concentration of 1 per cent for one hundred and ten days and in a concentration of 3 per cent for twenty days, in the drinking water. The renal calcium amounted to 819 mg. in 100 Gm. wet weight; the blood nonprotein nitrogen, to 76.2 mg. in 100 cc.

B, hemoglobin casts in renal tubules of a rabbit given 5 cc. per kilogram of propylene glycol intravenously and killed on the ninth day. The hemoglobin content was 40 per cent (Dare); the blood nonprotein nitrogen, 69.4 mg. in 100 cc.

C, vacuolation of cells of convoluted tubules and slight leakage of protein into glomerular spaces in the kidney of a rabbit that received 2 cc. per kilogram of diethylene glycol intravenously and was killed twenty hours later.

D, hydropic degeneration of convoluted tubules and distention of glomerular spaces in the kidney of a rat that consumed diethylene glycol in a concentration of 5 per cent in the drinking water. The animal became sick and was killed at the end of seventy-two hours.

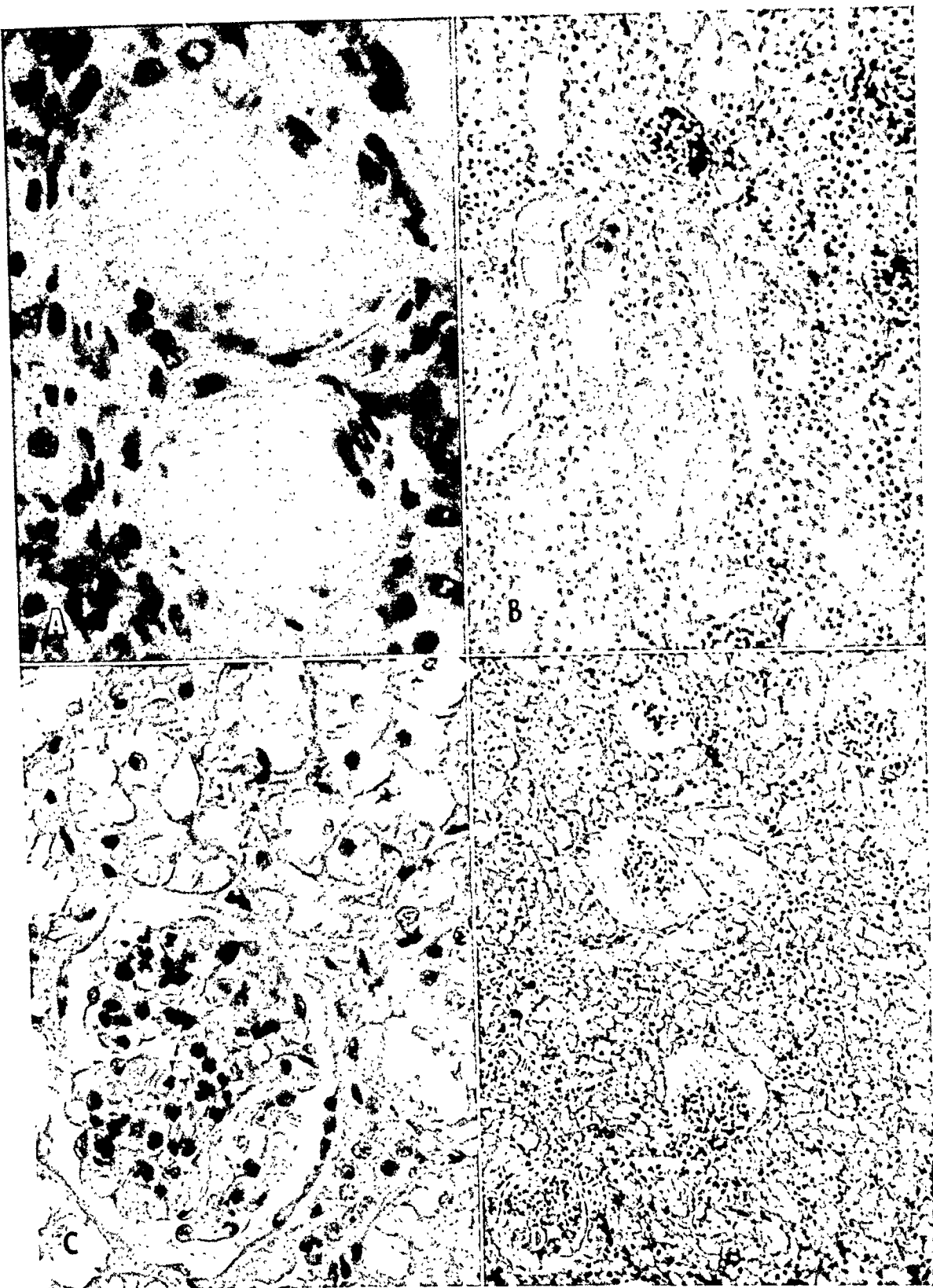


Figure 1

The 1,2 form of propylene glycol was administered to 39 rats in their drinking water in concentrations of from 1 to 10 per cent for from eleven to two hundred and thirty-four days with no demonstrable effect. Fifteen rabbits were given by intravenous injection single doses of from 2 to 5 cc. per kilogram of body weight. One rabbit that received a 5 cc. dose died the following night, but nothing abnormal was observed except the presence of considerable quantities of protein within the tubules of the kidneys. The other 14 rabbits were killed within from two to forty-six days after the injection. No pathologic changes were found in 10 of these.

Four rabbits which had been given intravenously 4 or 5 cc. of propylene glycol per kilogram of body weight and which were killed at the expiration of from two to twenty-three days exhibited in the kidneys evidence of the hemolytic action of the compound. This was visible grossly as reddish brown streaks and points on both the capsular and the cut surfaces. These discolorations consisted of hemoglobin-containing casts which could be found in all levels of the tubular system (fig. 1 B). Granular and hyaline casts were also sometimes present. Leakage of erythrocytes into glomerular spaces and convoluted tubules was sometimes seen. One of these rabbits which received a 5 cc. dose had 238 mg. of nonprotein nitrogen in 100 cc. of blood when killed forty-eight hours after the injection. In addition to all the aforementioned changes, small groups of renal convoluted tubules were necrotic, and occasional mitoses were found in adjacent remaining epithelial cells of this animal.

DIETHYLENE GLYCOL

Since the series of fatalities in October 1937 following the use of an elixir of sulfanilamide made with diethylene glycol as one of the solvents, several reports have appeared on the pathologic effects of large doses of diglycol on human beings and animals (Kesten, Mulinos and Pomerantz;⁷ Holck;^{6e} Cannon;⁸ Ruprecht and Nelson;⁹ Hagebusch;¹⁰ Lynch;¹¹ Cannon and Geiling¹²). Prior to that time only two studies of the toxicity of diglycol had been published (von Oettingen and Jirouch;¹³ Haag and Ambrose¹⁴), in neither of which were details of pathologic changes noted. Von Oettingen and Jirouch¹³ mentioned

7. Kesten, H. D.; Mulinos, M. G., and Pomerantz, L.: *J. A. M. A.* **109**: 1509, 1937.

8. Cannon, P. R.: *J. A. M. A.* **109**:1536, 1937.

9. Ruprecht, H. A., and Nelson, I. A.: *J. A. M. A.* **109**:1537, 1937.

10. Hagebusch, O. E.: *J. A. M. A.* **109**:1537, 1937.

11. Lynch, K. M.: *South. M. J.* **31**:134, 1938.

12. Cannon, P. R., and Geiling, E. M. K.: *J. A. M. A.* **111**:919, 1938.

13. von Oettingen, W. F., and Jirouch, E. A.: *J. Pharmacol. & Exper. Therap.* **42**:355, 1931.

14. Haag, H. B., and Ambrose, A. M.: *J. Pharmacol. & Exper. Therap.* **59**: 93, 1937.

the occurrence of acute nephrosis in rats that had been given subcutaneous injections of one or the other of the following: diethylene glycol, its ethyl ether, or carbitol; the ethyl ether of ethylene glycol, or cellosolve; cellosolve acetate; butyl cellosolve, and dioxane.

The effects of diglycol were studied as follows:

1. Seventeen rats received diglycol in 0.5 per cent concentration in the drinking water for from twenty-two to one hundred and twenty-four days. No deaths and no lesions occurred as a result of the procedure.

2. Thirty rats drank 1 per cent diglycol for from thirty-three to two hundred and seventeen days without any demonstrable effect.

3. Thirty rats received 3 per cent diglycol in the drinking water for from four to ninety-five days (approximately 3.5 cc. of diglycol per kilogram of body weight per day). Twenty-one died in from four to fifty-six days; hydropic degeneration of the renal cortex was present in 20. The livers of 13 of these contained foci of similar degeneration. The remaining 9 were killed at from fifty-one to ninety-five days. No lesions referable to the procedure were present.

4. Forty-two rats were on 5 per cent diglycol (approximately 6 cc. per kilogram of body weight per day) for from one to thirty-six days, of which 9 died in from one to six days. Hydropic degeneration had occurred in the kidneys of the 9 animals and in the livers of 5. Of the remaining 33, killed at from one to thirty-six days, similar damage of the kidneys was exhibited in 27 and degeneration of the liver in 13.

5. Six rabbits received from 1 to 4.6 cc. of diethylene glycol per kilogram of body weight in from 1 to 5 per cent concentration in the drinking water during from five to twenty-eight days. One rabbit which had consumed 1 cc. per kilogram died on the seventh day with extensive renal damage and edema of the lungs. Of 5 killed at from five to twenty-eight days, 4 presented lesions in the kidneys and 2 in the liver.

6. Thirty-five rabbits were given intravenous injections of from 1 to 2 cc. per kilogram of body weight, usually in a single dose, of full strength or 50 per cent diglycol (redistilled in a few instances). Eleven animals died in from one and a half to twenty-one days. The kidneys of 10 and the livers of 8 of them had undergone varying degrees of hydropic degeneration. The remaining 24 were killed after from one to forty-four days, and similar changes were observed in the kidneys of 15, and in the livers of 9. One instance was noted in which, following the injection of 2 cc. per kilogram of body weight, twenty hours was sufficient for the development of extensive changes in the kidneys, with a rise in the non-protein nitrogen of the blood to 86 mg. in 100 cc. (fig. 1 C).

7. Five guinea pigs received from 2 to 5 cc. per kilogram of body weight of diglycol by mouth during from two to twelve days. Two died at three and five days, respectively, with lesions in the kidneys similar to those in the rats and rabbits. One showed the liver similarly involved. The remaining 3 were killed at from two to twelve days. Hydropic degeneration had occurred in the kidneys of all and in the livers of 2.

Control animals on the same stock diet without the addition of diglycol remained normal.

Animals affected by diglycol become comatose and often anuric prior to death. For several hours following intravenous injection of diglycol

EXPLANATION OF FIGURE 2

A, calcification and regeneration of the convoluted tubules in the kidney of a rabbit that received 1 cc. per kilogram of diethylene glycol intravenously. The animal became sick and was killed on the ninth day. The blood nonprotein nitrogen amounted to 389 mg. in 100 cc.

B, hydropic degeneration of liver cells about efferent veins in a rabbit that received 2 cc. per kilogram of diethylene glycol intravenously. Death occurred in thirty-six hours.

C, hydropic degeneration of convoluted tubules of the kidney of a rat the drinking water of which contained 5 per cent methyl carbitol for twenty-eight days.

D, hydropic degeneration of convoluted tubules of the kidney of a rat which received butyl carbitol in a concentration of 5 per cent in the drinking water. It was killed on the eighth day. The blood nonprotein nitrogen was 162 mg. in 100 cc.

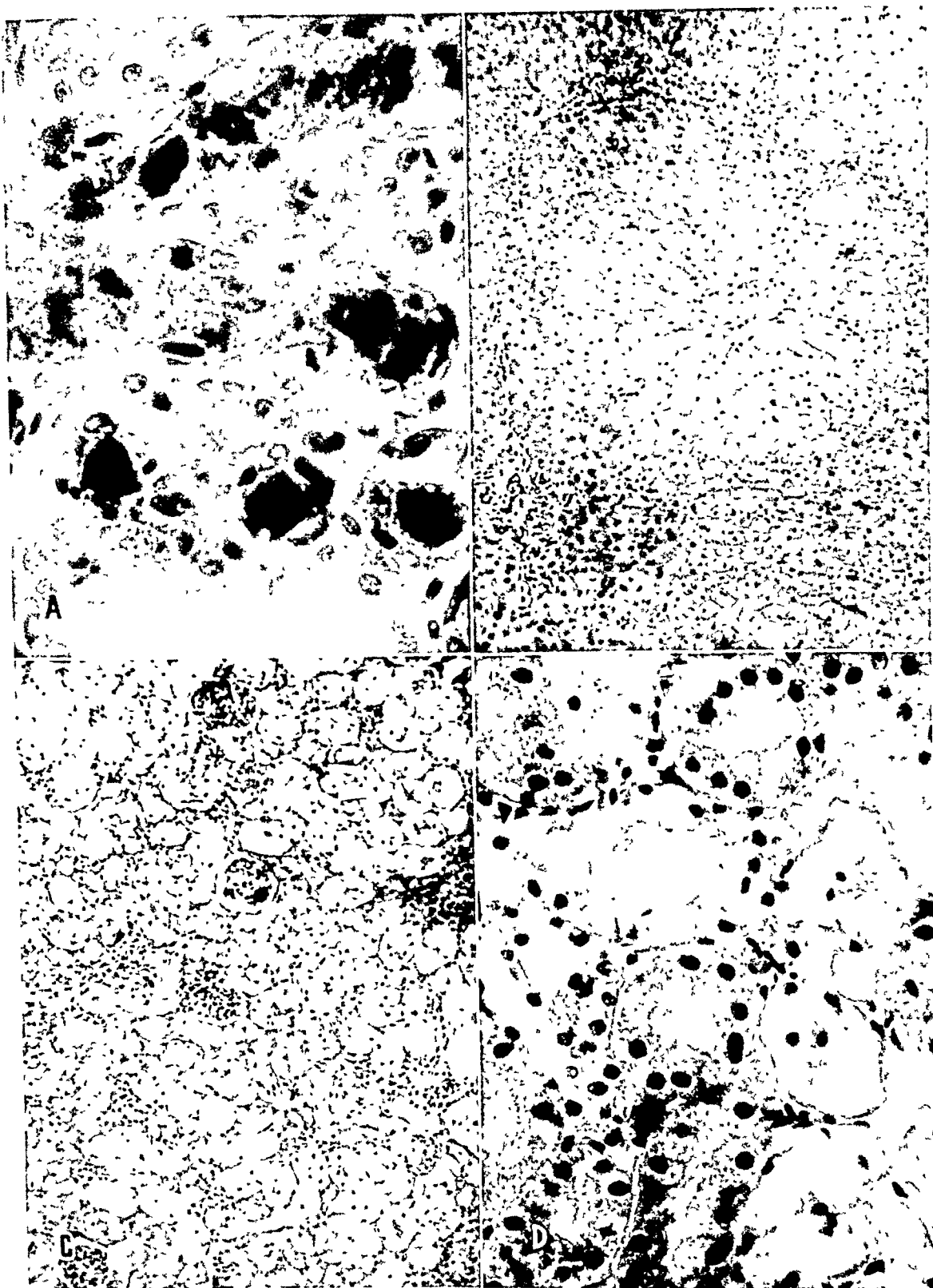


Figure 2

many of the rabbits pass smoky urine which reacts with benzidine. The nonprotein nitrogen of the blood in all the affected animals mounts steadily to from eight to ten times the normal amount (e. g., 389 mg. in 100 cc. in a rabbit that lived nine days after the injection of 1 cc. per kilogram of body weight).

The organs regularly affected in diglycol poisoning are the liver and kidney. The kidneys of the three species of animals used are swollen, pale yellowish tan and apparently more moist than normal. On analysis the water content is found to be increased approximately 6 per cent (to an average of 84 per cent from a normal average of 78 per cent). The cortical markings are indistinct. The medulla often appears to be congested. The outstanding histologic change is hydropic degeneration of the convoluted tubules. Every tubule may be and commonly is involved, or the change may be confined to isolated small groups of tubules. The epithelial cells swell and undergo coarse vacuolation, often with fusion of the vacuoles to a single large space that represents the entire cell save for an irregular cell membrane and a compressed pyknotic nucleus. The vacuoles are devoid of fat or glycogen, but occasionally a little finely divided fat is present in the remaining cytoplasm or in otherwise unchanged epithelial cells. Necrosis of the vacuolated cells occurs, and the tubular lumen becomes blocked by swollen cells and cell detritus. Obstruction to the outflow of urine ensues, leading to dilatation of the glomerular spaces. The glomerular tufts are often compressed and bloodless but otherwise are essentially uninvolved. Calcification of necrotic cytoplasm may set in as early as the fifth day in rabbits, and attempts at regeneration may be present, characterized by mitoses (fig. 2*A*). Leukocytic reaction is not a feature. Changes in the medulla are confined to congestion of the capillaries and small veins and to the occurrence of many hyaline and granular casts in the collecting tubules. Occasionally a little finely divided fat is found in the epithelium of such tubules.

The liver, though less frequently involved, may be enlarged and pale, and appear moist. An increase of approximately 10 per cent in volatile matter is observed (to an average of 82 per cent from a normal average of 72 per cent). The cells about the efferent vein in each lobule, often extending well out toward the portal areas, enlarge, and the cytoplasm becomes pale. Many tiny vacuoles appear in it, which fuse together until the cell consists of a single large vacuole surrounding a compressed and pyknotic central nucleus (fig. 2*B*). These vacuoles are also free from fat and glycogen, but in rare instances a little finely divided stainable fat is present in the other liver cells.

In a small percentage of the animals that have changes in the kidneys and liver, the epithelial cells of the outer fourth of the adrenal cortex

are swollen, pale staining and vacuolated in the same manner as the liver cells. The vacuoles do not contain stainable fat.

The lesions noted in the kidney and liver are essentially similar to those which have been described by other investigators¹⁵ coincident with and since the appearance of our preliminary report.⁷ The kidneys of animals, however, apparently do not contain the small arterial and capillary thromboses and massive cortical necrosis found in the kidneys of some of the human beings who were poisoned by large quantities of diglycol.¹⁶

ETHYL DIETHYLENE GLYCOL (CARBITOL)

Three ether derivatives of diethylene glycol were investigated briefly: ethyl diethylene glycol (diglycol monoethyl ether), or carbitol; methyl diethylene glycol (diglycol monomethyl ether), or methyl carbitol, and butyl diethylene glycol (diglycol mono-n-butyl ether), or butyl carbitol.

Carbitol is among the chemicals reported by von Oettingen and Jirouch¹⁵ as producing acute nephrosis in rats. A communication from the director of a pharmaceutical laboratory to the Council on Pharmacy and Chemistry of the American Medical Association¹⁷ stated that the equivalent of 1.67 cc. per kilogram of body weight of carbitol had been administered to rats daily for twenty days without apparent effect. One rat of the series which was put to death after sixteen days had normal kidneys and liver.

The drinking water of 6 rats was made up to 5 per cent concentration of carbitol, and the rats consumed varying amounts of it for from one to fifteen days. The intake was not over half the intake of drinking water in the preceding experiments, averaging approximately 15 cc. per day. Two rats died on the third and fifth days, respectively, and exhibited evidence of renal disease. Of the remaining 4, which were killed at the expiration of from one to fifteen days, 2, killed on the fifth day, had a nonprotein nitrogen concentration in the blood of 279 and 289 mg. in 100 cc., respectively, and exhibited lesions of the kidneys. Because of the difficulty encountered in obtaining an adequate intake of the material in the drinking water, an attempt was made to administer a solution to 2 rats by stomach tube, but this led to acute bronchitis and bronchopneumonia with death on the second and third days, respectively, without renal involvement.

A total of approximately 10 cc. of carbitol per kilogram of body weight had been consumed in the drinking water by the rat that died on the third day and that had lesions in the kidneys, and 15 cc. per kilogram by the one that died on the fifth day. However, the intake of fluid by the rats to which carbitol was offered was below normal, so that partial dehydration may have contributed to the result. Furthermore, many of the animals receiving carbitol,

15. Cannon.⁸ Ruprecht and Nelson.⁹ Hagebusch.¹⁰ Lynch.¹¹ Cannon and Geiling.¹²

16. Cannon.⁸ Ruprecht and Nelson.⁹ Lynch.¹¹ Cannon and Geiling.¹²

17. "Carbitol," Queries and Minor Notes, J. A. M. A. **110**:1692, 1938.

methyl carbitol and butyl carbitol refused food for some time before death, and a certain degree of starvation was commonly present. Such animals not infrequently showed pathologic changes associated with inanition, such as excessive hemosiderin deposits in the spleen, small hemorrhages and erosions in the mucous membrane of the alimentary tract, and enlargement of the adrenals.¹⁸

The kidneys of rats receiving carbitol undergo changes identical in every respect with those seen in animals on diethylene glycol itself, i. e., extensive hydropic degeneration of the epithelium of the convoluted tubules leading to obstruction. The other viscera examined were essentially normal.

METHYL DIETHYLENE GLYCOL (METHYL CARBITOL)

No experiments on the toxicity of this compound had been carried out previously.

Nine rats were given methyl carbitol in from 3 to 5 per cent concentration in their drinking water for from eleven to sixty-four days. (Four of these had previously been on 1 per cent methyl carbitol for one hundred and ten days without apparent effect.) One animal died, at the end of sixty-four days. Except for moderate amounts of protein in the lumens of the convoluted tubules of the kidney, its organs were normal. Of the 8 that were killed at the expiration of from eleven to forty-five days, the kidneys of 3 (twenty-eight, twenty-eight and forty-five days, respectively) had been damaged.

Methyl carbitol is also not well taken in the drinking water by rats. Two attempts to administer the compound by stomach tube were followed by acute bronchitis and pneumonia.

Methyl carbitol, ingested by mouth, produces damage in the kidneys of rats analogous to that produced by diglycol (fig. 2 C). Somewhat larger amounts of the methyl derivative than of carbitol itself are apparently necessary to produce these effects.

BUTYL DIETHYLENE GLYCOL (BUTYL CARBITOL)

Browning¹ refers to a factory worker exposed to either butyl carbitol or butyl cellosolve or both, in whom hematuria with casts developed. No animal experiments on the effects of butyl carbitol had been carried out previously.

The compound was administered in from 3 to 5 per cent solution in the drinking water of 9 rats for from five to thirty-five days. (Two of these animals had previously been on 1 per cent butyl carbitol for one hundred and nine days without apparent effect.) Three rats died on the fifth, twenty-third and thirty-fifth days, respectively. The kidney of the rat that died on the fifth day contained an occasional small group of damaged convoluted tubules. The kidneys of 2 of the remaining rats, killed at the expiration of from eight to

18. Jackson, C. M.: Effects of Inanition and Malnutrition upon Growth and Structure, Philadelphia, P. Blakiston's Son & Co., 1925.

sixteen days, had undergone similar changes. Butyl carbitol in from 3 to 5 per cent concentration, is relatively unpalatable to rats, leading to partial dehydration and partial starvation.

The renal lesions caused by this compound are similar to those following the administration of diethylene glycol but are much less extensive with the dosage used (fig. 2 *D*).

DIETHYLENE DIOXIDE (DIOXANE)

Dioxane, the second ether of ethylene glycol, contains two ether linkages in its molecule. It is a widely used solvent, and its toxicity has been extensively studied. The literature has been summarized by Browning.¹ Dioxane is in the series of compounds causing acute nephrosis studied by von Oettingen and Jirouch.¹⁹ Fairley, Linton and Ford-Moore¹⁰ described peripheral necrosis of hepatic lobules and necrosis of tubular epithelium in the kidneys of guinea pigs, rats and rabbits receiving the substance, although Yant, Schrenk, Waite and Patty²⁰ in an earlier series of acute inhalation experiments found no lesions of these organs in guinea pigs. Following a report by Barber²¹ of the death of 5 workers in the manufacture of artificial silk all of whom had central necrosis of the liver and symmetric necrosis of the renal cortex associated with vascular lesions, De Navasquez²² reinvestigated the effects of dioxane when given intravenously and intragastrically to rabbits, cats and guinea pigs. He observed acute hydropic degeneration of the convoluted tubules in the kidneys of all the animals, and in rabbits, vacuolation of the liver cells about the efferent veins, the vacuoles being apparently filled with glycogen.

Dioxane is highly toxic to rats. Of 10 animals given the compound in 5 per cent concentration in their drinking water, 8 died in from five to twelve days. The kidneys of the 8 rats were the site of extensive degeneration, and the livers of 5 were also abnormal. The kidneys of the remaining 2 animals, killed on the sixth and eighth days, respectively, were similarly involved. One of these also showed involvement of the liver. Two rats on 1 per cent dioxane for one hundred and ten days, followed by 3 per cent for forty-one and forty-eight days, respectively, had patchy areas of degeneration in the kidneys. One also had hepatic damage.

Five per cent dioxane is relatively unpalatable to rats and was poorly taken. The estimated intake of dioxane was 1 cc. per kilogram of body weight per day, which sufficed to cause fatal renal damage in five days—an observation which emphasizes the relatively high toxicity of this compound when taken by mouth.

19. Fairley, A.; Linton, E. C., and Ford-Moore, A. H.: *J. Hyg.* **34**:486, 1934.

20. Yant, W. P.; Schrenk, H. H.; Waite, C. P., and Patty, F. A.: *Pub. Health Rep.* **45**:2023, 1930.

21. Barber, H.: *Guy's Hosp. Rep.* **84**:267, 1934.

22. De Navasquez, S.: *J. Hyg.* **35**:540, 1936.

EXPLANATION OF FIGURE 3

A, hydropic degeneration of the renal cortex in a rat which received dioxane in a concentration of 5 per cent in the drinking water. Death occurred on the sixth day.

B, hydropic degeneration of liver cells about efferent veins in a rabbit which received 1.5 cc. per kilogram of dioxane intravenously and was killed on the fourth day. The blood nonprotein nitrogen amounted to 242 mg. in 100 cc.

C, hydropic degeneration of the renal cortex of a rabbit that received 4 cc. per kilogram of dipropylene glycol and was killed seventy-two hours later. The blood nonprotein nitrogen amounted to 286 mg. in 100 cc.

D, hydropic degeneration of the liver cells adjacent to an efferent vein in a rabbit that received 4 cc. per kilogram of dipropylene glycol intravenously and was killed at the expiration of seventy-two hours.

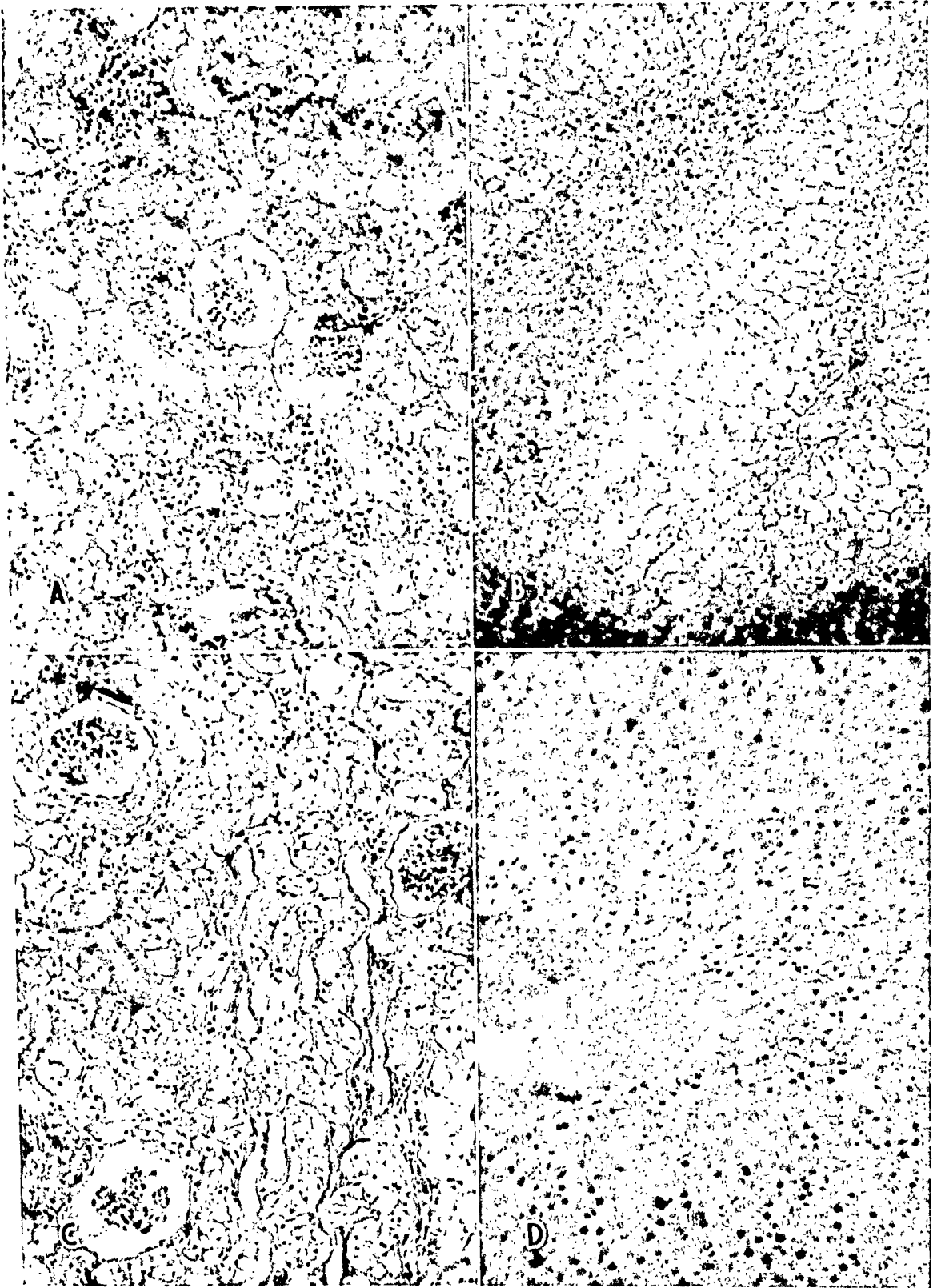


Figure 3

Two rabbits were given intravenous injections of 1.5 cc. of dioxane per kilogram of body weight in 25 per cent solution. One died two days later, and its kidneys exhibited several cortical areas of degeneration. The kidneys and liver of the second rabbit, killed on the fourth day, were markedly damaged, and the nonprotein nitrogen of the blood had risen to 242 mg. in 100 cc.

The pathologic observations in the kidneys of the animals are essentially those described by De Navasquez²² and by Fairley, Linton and Ford-Moore.¹⁹ They reproduce exactly the lesions due to diethylene glycol, namely, extensive hydropic degeneration of the epithelium of the convoluted tubules, every tubule being involved in most of the animals (fig. 3 *A*). High levels of blood nonprotein nitrogen ensue. Symmetric cortical necroses are not seen.

The livers of affected animals are also the site of the same lesion observed in the animals receiving diglycol, namely, swelling and hydropic degeneration of the liver cells about efferent veins (fig. 3 *B*). De Navasquez found the vacuoles in the liver cells to be filled with glycogen and was doubtful whether they could be considered pathologic. Although we were able to stain a little glycogen in some of the liver cells (by fixing thin slices in absolute alcohol immediately after death, embedding in pyroxylin, and treating with Best's carmine stain), this glycogen is finely divided or in a diffuse state and is in the cytoplasm present about the vacuoles. The vacuoles themselves remain empty looking and unstained. Fat is not present in them, nor is it present in the vacuolated cells in the kidney. A little finely divided fat is, however, occasionally seen in both the epithelial and the Kupffer cells of the liver.

DIPROPYLENE GLYCOL

The pathologic effects of dipropylene glycol, the next higher homologue of diethylene glycol, had not been studied previously.

The drinking water of 7 rats contained from 1 to 5 per cent of dipropylene glycol for from thirty-three to seventy-seven days, without detectable effect on the animals. All organs were normal. When, however, a concentration of 10 per cent was given over periods of from nine to sixty-eight days to a total of 25 rats, 7 died at the end of from ten to thirty days. The kidneys of 5 of the dead rats had become the site of lesions (as early as the tenth day in 1 rat). The remaining 18 rats were killed after from nine to sixty-eight days. The kidneys of only 4 of these had become involved (as early as the ninth day in 1).

Ten rabbits received from 2 to 4 cc. per kilogram of body weight of dipropylene glycol intravenously. Two of these rabbits died on the fourth day, and lesions had occurred in the kidneys of both. The remaining 8 were killed after from one to twenty-one days. The kidneys of 3 exhibited similar lesions (as early as the third day in a single instance). One of these also had involvement of the liver. A dose of 4 cc. per kilogram was necessary to cause renal changes and to raise the nonprotein nitrogen of the blood (to as high as 406 mg. in 100 cc. on the sixth day in 1 rat).

Lesions identical to those produced by diethylene glycol are present in animals receiving dipropylene glycol. Extensive hydropic degeneration of renal epithelium is seen, with, in some cases, an occasional hemoglobin-containing cast in the collecting tubules (fig. 3 C). Damage to liver parenchyma is more inconstant and more difficult to produce (fig. 3 D).

TABLE 2.—*Summary of Protocols*

Compound	Animals	Route of Administration	Amount	Duration, Days	Observations in	
					Kidney (Nature of Lesion; Percent- age of Animals Affected)	Liver (Hydropic Degeneration; Percent- age of Animals Affected)
					Oxalate crystals	
Ethylene glycol; glycol...	30 rats	Mouth	1-5%	8-79	67	0
	11 rabbits	Vein	1-4 cc. per Kg.	1-120	36	0
Ethylene glycol diacetate	11 rats	Mouth	1-5%	7-130	73	0
					Hemoglobin casts	
Propylene glycol.....	39 rats	Mouth	1-10%	11-234	0	0
	15 rabbits	Vein	2-5 cc. per Kg.	1-46	27	0
					Hydropic degeneration	
Diethylene glycol; digly- col	47 rats	Mouth	0.5-1%	22-217	0	0
	72 rats	Mouth	3-5%	1-95	78	43
	6 rabbits	Mouth	1-5%	5-28	83	33
	35 rabbits	Vein	1-2 cc. per Kg.	1-44	71	49
	5 guinea pigs	Mouth	2-5 cc. per Kg.	2-12	100	60
Ethyl diethylene glycol (diglycol monoethyl ether); carbitol	6 rats	Mouth	5%	1-15	67	0
Methyl diethylene glycol (diglycol monomethyl ether); methyl carbitol	9 rats	Mouth	3-5%	11-64	33	0
Butyl diethylene glycol (diglycol mono-n-butyl ether); butyl carbitol	9 rats	Mouth	3-5%	5-35	33	0
Diethylene dioxide; dioxane	10 rats	Mouth	5%	5-12	100	60
	2 rabbits	Vein	1.5 cc. per Kg.	2-4	100	50
Dipropylene glycol.....	7 rats	Mouth	1-5%	33-77	0	0
	25 rats	Mouth	10%	9-68	36	0
	10 rabbits	Vein	2-4 cc. per Kg.	1-21	50	10

COMMENT

The several experiments are summarized in table 2. From them there emerge the following observations: (a) ethylene glycol and ethylene glycol diacetate may cause deposition of oxalates in the kidneys and mild renal insufficiency; (b) propylene glycol is relatively harmless, especially when given by mouth, large intravenous doses being necessary to cause hemoglobinuria and casts; (c) diethylene glycol, the methyl, ethyl and butyl ethers of diethylene glycol, dioxane and dipropylene glycol are all capable of causing profound degeneration of renal epithelium leading to uremia.

The members of this last group of compounds have at least one structural factor in common. The molecules of diglycol and its ethers, of dipropylene glycol and of dioxane contain an ether linkage between two glycol molecules. The cyclic compound dioxane possesses two such linkages, which may in part account for its relatively high toxicity. The molecules of ethylene glycol, ethylene glycol diacetate and propylene glycol lack this particular type of ether linkage. At the same time their respective effects on parenchymatous epithelium differ from those of the preceding compounds. It is suggested that the ether linkage between two glycol molecules may be responsible for the hydropic degeneration of the epithelium of the convoluted tubules and of the liver cords.

An outstanding feature of the effect of the compounds causing hydropic degeneration of the kidney is the extensive character of the damage wrought. In each experiment one or more of the animals was apparently unaffected by the drug, but in the injured animals the lesions were usually maximal, involving as a rule the entire cytoplasm of every cell in every convoluted tubule. Occasionally only groups of tubules were the site of degeneration, but almost invariably every cell of the segment of tubule involved was completely hydropic. Multiple small vacuoles in a given cell were uncommon. It would seem that the colloidal structure of the cytoplasm of an injured cell relatively suddenly loses its integrity and approximates a true solution.

The animals that received diglycol and its ethers, dioxane and dipropylene glycol died usually as a result of the damage to the convoluted tubules, in uremia. The carbitols and dipropylene glycol are not palatable to rats in moderate concentrations, so that some of these animals consumed less water than their minimal requirements in view of the dry nature of their diet. Partial refusal of food sometimes followed, and dehydration and starvation were probably factors in the death of certain of these animals. The cause of death of the animals that received glycol and glycol diacetate was not always obvious. In not all instances were the deposits of oxalates in the kidney sufficiently extensive to cause demonstrable obstruction of tubules and a significant rise in the nonprotein nitrogen of the blood. As suggested by Hunt ^{6a} a question arises as to the possible formation of toxic intermediary products.

The relative toxicity of these compounds is roughly indicated in the protocols and will be taken up in detail in a separate report. It may be emphasized, however, that appreciable amounts of the several chemicals must be given to produce demonstrable changes in the animals used. In no instance was less than 1 cc. per kilogram of body weight effective.

CONCLUSIONS

Dipropylene glycol, diethylene glycol, dioxane, carbitol, methyl carbitol and butyl carbitol when administered in adequate dosage orally or intravenously to animals cause extensive hydropic degeneration of the renal convoluted tubules, leading to uremia; less regularly, there is hydropic degeneration of the liver parenchyma. These compounds resemble each other in containing an ether linkage between glycol molecules. Ethylene glycol, ethylene glycol diacetate, and propylene glycol do not have such a structure, nor do they cause similar lesions in animals.

CARTILAGINOUS FOCI IN THE HEARTS OF WHITE RATS AND OF MICE

W. C. HUEPER, M.D.

NEW YORK

Spontaneous cardiovascular lesions in small rodents used for experimental purposes have been reported recently (Hueper¹; Wilens and Sproul²). No mention has been made of cartilage in the hearts of rats and mice as described in this report.

During routine histologic examination of several thousand hearts of white rats and mice small cartilaginous nodules or bands in the region of the aortic ring were observed in a number of animals, which were as a rule young adults. As no systematic study was made, accurate figures for the incidence of the cartilagenous formations and for their relation to age are not available. The hearts were usually cut longitudinally through the base and apex. The presence or absence of the aortic bulb in the sections determined mainly the chance of finding cartilaginous areas. They were seen in about 100 of approximately 3,000 rats and in about 15 of 1,000 mice. The actual incidence probably was much higher.

There are usually two cartilaginous nodules in the aortic ring, one at the site of attachment of the right semilunar valve and the other at the base of the left semilunar valve. It is not rare that a sort of transitional tissue or even well developed cartilage connects the two nodes in the form of a curved band. The nodules are irregular in shape and indistinctly defined. In their central portions they consist of a hyaline cartilage, while in the marginal parts they contain some fibrillar material, form small cartilaginous buds and gradually merge with the surrounding fibroblastic, myxomatoid or muscular tissue. The cartilaginous cells are embedded in a bluish-staining matrix. They are often vesicular or seem to have undergone mucoid transformation. As shown in the figure, the cells contain not infrequently two oval or round nuclei, located in small cavities.

It is obvious that these cartilaginous deposits in the fibrous skeleton of the heart are not the products of a metaplastic process, such as that observed occasionally in degenerative foci of the heart and arteries of

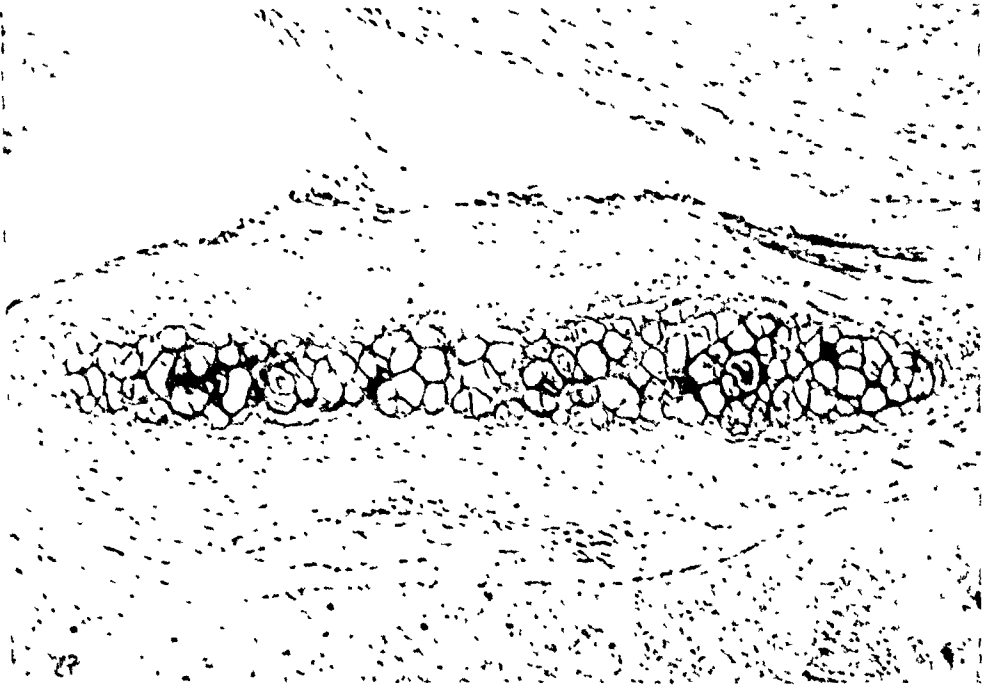
From the Warner Institute for Therapeutic Research.

1. Hueper, W. C.: *Arch. Path.* **20**:708, 1935.

2. Wilens, S. L., and Sproul, E. E.: *Am. J. Path.* **14**:177 and 201, 1938.

man and animals (Ribbert³; Mönckeberg⁴; Weizmann⁵; Spiegel⁶; Miesowicz⁷; Otto⁸; Lillie⁹) but are the results of a normoplastic transformation of fibrous tissue exposed to special stress under physiologic conditions.

These cartilaginous foci are comparable to similar ones in the hearts of mammals (horse, sheep, buffalo, rabbit), reptiles (turtle, alligator, crocodile) and birds (chicken) (Retterer and Lelièvre¹⁰; Kern¹¹; Hausotter¹²; Favaro¹³; Greil¹⁴; Beddard and Mitchell¹⁵; Stiefel¹⁶;



Cartilaginous plate in the aortic ring of the heart of a rat.

3. Ribbert, H.: *Lehrbuch der allgemeinen Pathologie und der vergleichenden Anatomie*, Leipzig, F. C. W. Vogel, 1923.
4. Mönckeberg, T. G.: *Virchows Arch. f. path. Anat.* **167**:191, 1902.
5. Weizmann, M.: *Systematische histologische Untersuchungen über den Ductus resp. das Ligamentum Botalli im Anschluss an einen Fall von Verknorpelung des letzteren*, Inaug. Dissert., Zurich, A. Schereschewsky, 1911.
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7. Miesowicz, E.: *Zentralbl. f. allg. Path. u. path. Anat.* **18**:8, 1907.
8. Otto, C.: *Virchows Arch. f. path. Anat.* **203**:352, 1911.
9. Lillie, R. D.: *Arch. Path.* **18**:710, 1934.
10. Retterer, E., and Lelièvre, A.: *Compt. rend. Soc. de biol.* **72**:371 and 390, 1912.
11. Kern, A.: *Morphol. Jahrb.* **58**:125, 1927.
12. Hausotter, E.: *Wien. tierärztl. Monatschr.* **11**:311, 1924.
13. Favaro, G.: *Atti mem. r. Accad. di sc., lett. ed arti* **28**:2, 1912.
14. Greil, A.: *Morphol. Jahrb.* **31**:125, 1903.
15. Beddard and Mitchell, cited by Greil.¹⁴
16. Stiefel, K.: *Anat. Anz.* **61**:177, 1926.

Benninghoff ¹⁷). I can confirm by personal observations the report that such nodules occur in the hearts of rabbits (Vanzetti ¹⁸), studied during the same period as the rats and mice. In all instances the cartilage is hyaline. In sheep it may change into bone with increasing age through enchondral ossification. In birds it has been found not only in the aortic ring but also to a minor extent in the annulus of the pulmonary artery.

SUMMARY

Attention is called to the not infrequent occurrence of small cartilaginous areas in the aortic ring in white rats and mice which are not pathologic lesions but physiologic formations.

17. Benninghoff, A.: Das Herz, in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1930, vol. 6, pt. 1, p. 187.

18. Vanzetti, F.: Arch. ital. de biol. **56**:265, 1911.

SPIROCHETES IN THE GASTRIC GLANDS OF MACACUS RHESUS AND OF MAN WITH- OUT RELATED DISEASE

JAMES L. DOENGES, M.D.

ST. LOUIS

The observation made by Cowdry and Scott¹ that spirochetes occur in the gastric glands of normal-appearing monkeys of the species *Macacus rhesus* is of interest, for these animals are closer to man than dogs, cats and rats, in which spirochetes have been reported to occur in the same location, without any accompanying signs of illness.² For reasons difficult to explain, these spirochetes can be easily seen in ordinary sections stained with hematoxylin and eosin.

Data on spirochetes in the human stomach are less definite than data on spirochetes in the stomachs of animals. Escherich³ described spirochetes in the feces of patients with cholera and his observations have been confirmed. Le Dantec⁴ called attention to their occurrence in the feces of patients with diarrhea of long standing, which he did not believe to be cholera. With the introduction of arsenicals specific for syphilis, the diagnosis of spirillar dysentery was established.

Spirochetes in the human stomach were first reported by Salomon^{2b} in 1896. Confirmation followed, and it was emphasized that spirochetes were seen in the human stomach only in the presence of gastric carcinoma and only when the carcinoma was ulcerating. Their number was said to be proportional to the extent of the ulceration. Hoffmann⁵ stated that the spirochetes do not enter the tissue but are limited to the necrotic material at the surface. They were found by Krienitz⁶ in gastric contents removed by evacuation. Attempts to establish an etiologic relation between spirochetes and non-neoplastic gastric ulcers have not been successful. Some investigators have gone so far as to state that spirochetes are never found in them.⁷ Luger⁸ explained the presence of spirochetes in ulcerating carcinomatous areas and their absence in ulcers as due to the liberation in the former of proteins and amino acids which stimulate their growth.

From the Anatomical Laboratory, Washington University School of Medicine.

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2. (a) Bizzozero, G.: *Arch. f. mikr. Anat.* **42**:82, 1893. (b) Salomon, H.: *Centralbl. f. Bakt. (Abt. 1)* **19**:433, 1896.

3. Escherich, T.: *München. med. Wchnschr.* **15**:408, 1884.

4. Le Dantec: *Compt. rend. Soc. de biol.* **55**:617, 1903.

5. Hoffmann: *Berl. klin. Wchnschr.* **28**:550, 1905.

6. Krienitz, W.: *Deutsche med. Wchnschr.* **22**:872, 1906.

7. Celler, H. L., and Thalheimer, W.: *J. Exper. Med.* **23**:791, 1916. Rose-
now, E. C., and Stanford, A. H.: *J. Infect. Dis.* **17**:219, 1915.

8. Luger, A.: *Wien. klin. Wchnschr.* **52**:1643, 1917; *Ztschr. f. klin. Med.* **92**:54, 1921.

As to the nature or source of the spirochetes, little is known. Macfie and Carter⁹ described their occurrence in the stools of 56.2 per cent of normal persons. Whether those in the stools and stomach are identical is not known; but the consensus seems to be that they are neither the spirochetes of Vincent's angina nor those of syphilis.

Investigations on animals and on man have been but inadequately correlated. To obtain material from normal persons is possible only in cases of accidental death. In such cases tissues have apparently not been examined for spirochetes. In a preliminary note on specimens obtained at autopsy I have reported that spirochetes inhabit the gastric glands and invade the parietal cells in somewhat the same way as they do in monkeys.¹⁰ The main question is: Are human stomachs, like those of monkeys, dogs, cats and rats, regularly infected with spirochetes in the complete absence of definite related disease? If so, what is the incidence of spirochetes in human stomachs and how closely do these spirochetes resemble those in the stomach of *Macacus rhesus* in respect to location, associated lesions, if any, and other particulars?

An attempt has been made to secure this information by detailed study of sections of human stomachs removed at autopsy and of preparations of stomachs from monkeys.

OBSERVATIONS

From a large series, sections of 242 human stomachs were selected on the basis of good preservation and absence of marked postmortem changes, which it was thought gave opportunity for the discovery of spirochetes if they were present. The sections were stained with hematoxylin and eosin. Spirochetes were found in 103, or 43 per cent, of the specimens examined (fig. 1). It is likely that if more sections of each stomach had been studied the incidence of spirochetes would have been found to be greater. It was not possible to correlate their presence with the anatomic diagnosis. The 139 stomachs in which spirochetes were not found did not appear to differ in any significant way from the others.

Usually the spirochetes were few and were detected only after careful search. Sometimes granules were observed in the parietal cells resembling those observed by Edkins¹¹ in cats which had not been fed for some time. Perhaps the stomachs in question were from persons who had not taken nourishment for several hours or days before death. Eleven of the stomachs showed many spirochetes, but the infection was never so extensive as the maximum seen in the stomachs of the monkeys.

Four forms of spirochetes were seen in human gastric glands. The first two, indistinctly shown in figure 1, looked like those found in the monkey. One of these was a rather thick organism with only two or three spirals. The length varied from 4 to 8 microns, and the usual diameter was about 1 micron. This form was occasionally seen within parietal cells and was the one most frequently encountered in the human stomach. The two other forms were seen infrequently but at times were the only ones detected within a single section. Generally two of the varieties were found in the same section. The second most common spiro-

9. Macfie, J. W. S., and Carter, H. F.: *Ann. Trop. Med.* **11**:75, 1917.

10. Doenges, J. L.: *Proc. Soc. Exper. Biol. & Med.* **38**:536, 1938.

11. Edkins, J. S.: *Parasitology* **15**:296, 1920.

chete in the human stomach was an organism which in appearance was entirely similar to that already described but smaller in all dimensions. The length varied from 2 to 4 microns, and the average diameter was less than 0.5 micron. This spirochete also had only two or three spirals. The third form was discovered in only a few stomachs. Its length was between 8 and 20 microns, but it had only from three to five spirals. In diameter it was thin, being never more than 0.75 micron and usually not more than 0.5 micron. In 2 specimens spirochetes which showed sharp angulation were found. These were never abundant. This fourth form was about 6 microns in length and 0.75 micron in diameter and it had from six to eight spirals, which were equidistant and showed great regularity of shape. A similar spirochete was frequently seen in monkeys and is well shown in a parietal cell a little above and to the left of the center in figure 2 B.

The series of specimens from monkeys was smaller, but the tissues were removed immediately after the animals were killed and as nearly as possible from the same fundic region of the stomach. Most of them were preserved in



Fig. 1.—Spirochetes within the lumen of a human gastric gland. Note the cellular infiltration in the surrounding tissue fluid; $\times 2,000$.

formaldehyde-Zenker solution,¹² but several were fixed in a fluid that was 9 parts absolute alcohol and 1 part solution of formaldehyde U. S. P. The sections were stained with hematoxylin and eosin. No difference depending on the fixative used was observed in the appearance of the spirochetes.

Spirochetes were noted in all of 19 monkeys given viosterol, as detailed by Cowdry and Scott,¹ and in all of 24 others which were used as controls. No consistent difference was found between the spirochetes of treated and those of untreated animals. As a rule the spirochetes were very numerous, and only in an occasional section was difficulty experienced in finding them. They occurred at all depths of the gastric mucous membrane, sometimes in the mucous lining of its internal surface and always in the necks of the glands. In the latter situation the spirochetes often appeared as deeply stained plugs of material, suggestive of extracellular multiplication (fig. 2 A). The row of epithelial cells on

12. This is a fixing fluid made by substituting neutral solution of formaldehyde U. S. P. for glacial acetic acid in the original formula of Zenker's solution.

EXPLANATION OF FIGURE 2

A, large masses of spirochetes in the lumen of the neck of a gastric gland of a monkey. Observe the rarefaction of the distal cytoplasm covered by the spirochetes; $\times 2,000$.

B, spirochetes within the glandular lumen and parietal cells of a monkey. The number of spirals can be counted in two spirochetes in a parietal cell a little above and to the left of the center. In a parietal cell with a large nucleus near the upper right hand corner a spirochete is shown partly in the lumen and partly in the cytoplasm or in a canaliculus entering the cytoplasm from the lumen. Below this organism are at least 2 others within the same cell and surrounded by thin halos; $\times 2,000$.

C, numerous spirochetes within the lumen of a monkey's gland, limited chiefly by parietal cells, the acidophilic granular cytoplasm of which is well shown. The lumen extends from the upper left to the lower right. Examining the parietal cells from above downward on the left side of the lumen, the first 2 are of normal size and appearance. The nucleus of the second even possesses a well formed nucleolus. The nuclei of the next 3 cells are by contrast smaller, blurred, irregular and somewhat fragmented. Either the spirochetes extend into the lowermost parietal cell or the cytoplasm of the cell partly surrounds the glandular lumen. The background of these spirochetes looks like the luminal background higher up in the photomicrograph; $\times 2,000$.

D, lumen of a monkey's gastric gland. This lumen is not so straight as that in *C*. Entering the area from the right, it extends horizontally about half way across and then dips sharply downward. Above the first part of the horizontal portion is a binucleated parietal cell. In the cytoplasm to the left of the 2 nuclei is a short spirochete (or part of a long one) extending almost vertically upward and surrounded by a rather wide halo (or segment of a canaliculus). Next, on the same horizontal level, are the nuclei of 3 cells in which the cytoplasm is of small volume. These may be chief cells. Below the nucleus most distant from the right margin of the figure there is a suggestion of a spirochete. Passing now to the cells below the horizontal part of the lumen, one notes that the first 2 nuclei resemble those of chief cells, but there is a slight dip in the lumen with a definite and characteristic convergence of spirochetes into it, as if they were entering a parietal cell. The third nucleus from the right appears in section to be smaller and may belong to a cell in the tissue fluid. Extending from it to the left margin of the figure is granular cytoplasm, typical of parietal cells. The lateral cell walls cannot be distinguished, and the nuclei are faint and indistinct. The second nucleus from the left hand margin appears to have been fixed in the process of breaking up. In the lowest part of the dip of the glandular lumen, in the lower left portion of the figure, the spirochetes are oriented roughly parallel to each other and are partly within degenerating parietal cells; $\times 2,000$.

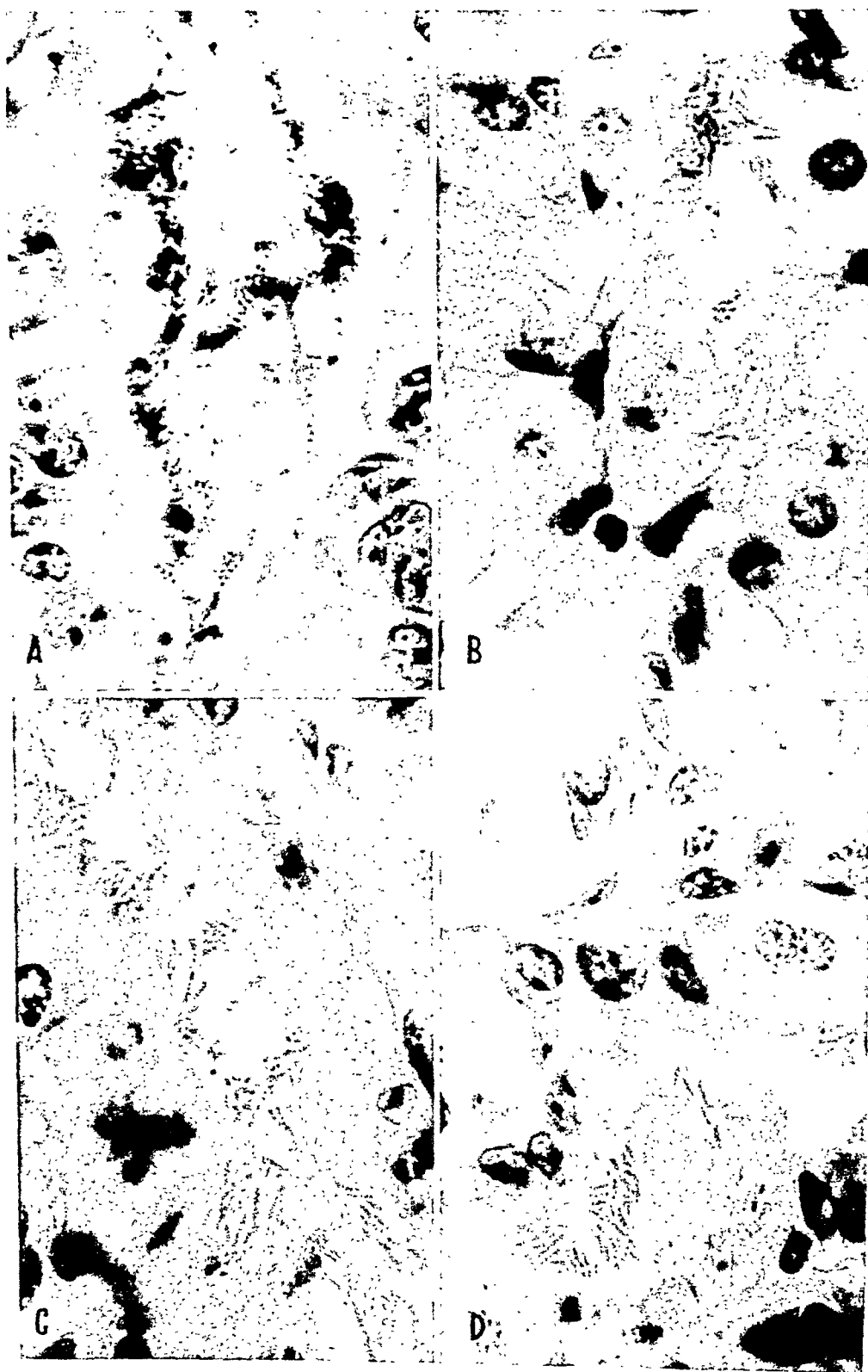


Figure 2

the left side of the lumen in figure 2*A* showed an interesting change. Their cytoplasm was divisible into two parts: an inner (proximal) and an outer (distal) part next the organisms. The latter appeared clear, as if the cytoplasm had been slightly altered by the plug of spirochetes. In other cases the cells next the spirochetes seemed to be flatter than usual. When comparatively few organisms were present in the lumens of the necks of the glands, many were found in the lumens of portions of the glands rich in body chief cells and parietal cells.

Though the majority of spirochetes were extracellular, a considerable number were intracellular, always in the parietal cells and never in goblet mucous cells or in pepsin-forming chief cells. As many as 7 were found in a single chief cell, oriented in general with their lengths parallel to its proximodistal axis. Some were seen next to the nucleus, while others were gathered about it. Signs of injury in infected chief cells were limited to the distal cell membrane and adjacent cytoplasm, when masses of spirochetes in the lumen were packed closely against it, and even then the signs were inconspicuous. The nuclei of such cells stained in the usual fashion.

In all the specimens from the monkeys spirochetes were noted within the parietal cells (fig. 2*B*), but the number in them did not appear to be in direct relation to the massive plugs of spirochetes in the lumens of the necks of the glands. Moreover, there were always more parietal cells infected than chief cells. The cell mainly involved in man, as well as in monkeys and in the other species described in the literature, is the parietal cell. In monkeys the number of organisms within a single parietal cell was generally between 2 and 8 but varied from 1 to 30. Often the entire cell was not included in a single section.

The exact position of the spirochetes within the monkey parietal cell was difficult to determine, because in hematoxylin-eosin preparations the outlines of the intracellular canaliculi were not clearly visible. It is to be remembered that Regaud¹³ came to the conclusion, from the study of sections treated with the iron-hematoxylin stain, that the spirochetes of cats and dogs occur only within the canaliculi and not freely in the cytoplasm of parietal cells. The only way to settle the matter of the position in monkeys would be to stain supravitaly the contents of the canaliculi by the methods of Harvey and Bensley¹⁴; but no living monkeys were available for the purpose. All that can be said is that in the hematoxylin-eosin specimens examined many spirochetes were present in the rather wide opening by which the canalicular system discharges into the lumen of the gland. Others were undoubtedly present within canaliculi situated more deeply in the substance of the parietal cells. Occasionally a single spirochete was observed in the cytoplasm surrounded by a faint halo (fig. 2*B*). It was not possible to determine whether the halo represented localized liquefaction of cytoplasm or a narrow canaliculus. In figure 2*B* there are two long spirochetes in a parietal cell above and a little to the left of the center. These appeared to be in close contact with the acidophilic granules. They were not throughout their lengths within recognizable canaliculi. Whether within the canaliculi or outside of them, the organisms tended to be arranged with their lengths in a proximodistal direction, but less distinctly than in the chief cells, for the parietal cells are of a more rounded shape than the chief cells. Frequently spirochetes were found apparently touching the nucleus or close to it. In general, the intracellular spirochetes occurred separately and were not closely applied together in masses of 4 or more.

13. Regaud, C.: *Compt. rend. Soc. de biol.* **1**:229, 1909.

14. Harvey, B. C. H., and Bensley, R. R.: *Biol. Bull.* **23**:225, 1912.

While all the chief cells escaped definite injury by the spirochetes except, perhaps, for slight rarefaction of the cytoplasm, which did not seem to be lethal, the parietal cells showed gradations between no apparent injury and death. The majority were not noticeably affected. In some the spirochetes were discovered in particularly close association with the nuclei, and in others they actually entered the nuclei, but this was a rare phenomenon. Some of the nuclei became shrunken, pyknotic and broken up (fig. 2 *C* and *D*). The cytoplasm became granular or vacuolated, the distal cell membrane was destroyed, and the cell substance exuded into the glandular lumen. The whole cell was sometimes cast out in this manner.

The tissue fluid just beneath (proximal to) the glandular epithelial cells was in some specimens invaded by leukocytes, mostly neutrophils and lymphocytes. A considerable number of plasma cells were seen. Though this invasion was sometimes associated topographically with cellular injury and an abundance of spirochetes, there were instances of its occurrence apart from the possibility of direct spirochetal injury, and in some glands heavily infected with spirochetes (fig. 2 *C*, to the right) the tissue fluid did not exhibit an unusual number of infiltrating cells.

It is to be emphasized that in the stomachs of both man and monkey the infection was far from uniform; one gland frequently contained many spirochetes and neighboring ones few, if any. Even in the same gland the parietal cells were affected individually, not *en masse*. Often normal-appearing cells, devoid of spirochetes, were in contact with severely injured ones.

Since all of the "infestations" occurred naturally, it was not feasible to establish a sequence of changes. There is, therefore, no evidence as to whether the spirochetes caused the injury of parietal cells or simply invaded parietal cells rather generally, some of which were near the end of their life cycle anyway and destined to disintegration and removal to make place for others.

In the monkey as in man fairly definite forms of spirochetes were observed. The first form is illustrated in figure 2 *B*, *C* and *D* but particularly in figure 2 *B*. It varied in length from 5 to 8 microns and in diameter from 0.5 to 1 micron (average 0.75). The number of sharply angular spirals varied from four to fourteen (average, six to nine). The second (fig. 2 *A*) was generally between 4 and 8 microns in length and 1 micron in diameter. It possessed of from two to four irregular and but slightly angular spirals and resembled closely the human spirochetes in figure 1. The third was a long slender organism about 0.5 micron in diameter and with only from two to four spirals in a length of 10 microns or more. It was seen only in the stomach of a single monkey. As a rule only one form was observed in a single gland. When two forms were seen in a section, there was always a great numerical predominance of one over the other.

COMMENT

The incidence of spirochetes in the gastric glands of *Macacus rhesus* was 100 per cent in a series of 43 animals. If the conditions for examination of human gastric glands had been more favorable, the incidence would probably have been found to be higher than 43 per cent. Bizzozero^{2a} found them in all dogs which he examined. Regaud¹³ considered them to be normal findings in both dogs and cats. Lim¹⁵ looked on spirochetes in the gastric glands of cats as a more or less

15. Lim, R. K. S.: *Parasitology* 12:108, 1920.

localized laboratory infection. Edkins¹¹ examined 105 cats from different localities and stated that it seemed permissible to regard spirochetes as just as characteristic of their gastric glands as fleas of their fur. Data for the incidence in rats¹⁶ are less definite. In general, however, it can be said that the reported incidence is either 100 per cent or approximately so in the various species most carefully studied. It is significant also that the infection is species specific. Though present in rats, the spirochetes are absent in mice (Kasai and Kobayashi¹⁷), and though present in *Macacus rhesus*, they are apparently lacking in *Cebus fatuellus* (Cowdry and Scott¹). Another point is that if they are pathogenic at all their pathogenicity is low. Kolmer and Wagner¹⁸ looked particularly for associated lesions and stated that hyperemia and leukocytosis were absent. It is also clear that the infection centers on a particular type of cell—the parietal cell, which is concerned with the formation of hydrochloric acid. It does not ordinarily extend to the intestine. For these reasons, the conclusion appears to be justified that *Macacus rhesus*, and probably man also, is to be listed with the dog, the cat and the rat as a species which harbors spirochetes in the gastric glands without definite clinical symptoms and further that because of the high incidence, lack of response by the host and restricted location in cells the infection in each species is likely to be one of long standing. No evidence is available as to the age when the infection begins. Cowdry and Scott¹ found spirochetes in monkeys about 2 years old as well as in others at least 10 years old.

An important question is: Can these spirochetes, which exist in man and in the animals commonly employed for experiments and which are ordinarily harmless, be ignored in physiologic and pathologic studies on the stomach? Information on which to base an answer is unfortunately rather inconclusive and scarce. Reference has been made to Edkins' observation that in cats the spirochetes are most abundant shortly after feeding and that as the hours pass fewer spirochetes are seen even in the parietal cells, in which he found small rounded bodies disposed roughly in spiral lines suggestive of a stage in multiplication. Cowdry and Scott¹ noted no difference in spirochetal infection of *Macacus rhesus* after administration of viosterol over both long and short periods. Oshikawa¹⁸ observed in gastroenterostomy of dogs that the spirochetes remained confined to mucosa containing parietal cells and did not spread to the anastomosed intestinal mucosa. Kasai and Kobayashi¹⁷ inoculated rabbits, which are naturally devoid of gastric spirochetes, with gastric spirochetes from cats and fixed rabies virus

16. Kolmer, W., and Wagner, R. J.: *Centralbl. f. Bakt. (Abt. 1)* **78**:382, 1916.

17. Kasai, K., and Kobayashi, R.: *J. Parasitol.* **6**:1, 1917.

18. Oshikawa: *Arch. f. klin. Chir.* **124**:559, 1923.

and inoculated a control group with spirochetes only. In the stomachs of the former punctate hemorrhages and hemorrhagic erosions resulted. In the control rabbits no similar lesions occurred, but large numbers of spirochetes were found in the stomachs of both groups of animals.

SUMMARY

Spirochetes were found in the gastric glands of 103 (43 per cent) of 242 human stomachs examined in the routine of autopsies. They were also noted in all of 19 monkeys (*Macacus rhesus*) treated with viosterol and in 24 left untreated. In both the human and the monkey stomachs the spirochetes occurred in the glandular lumens and in less number in the parietal cells. Distinctive clinical symptoms were absent, and microscopic lesions associated with the spirochetes were slight or altogether lacking.

PULMONARY EPITHELIUM AND PROLIFERATIVE REACTIONS IN THE LUNGS

A STUDY OF THE CELLULAR RESPONSE IN LUNGS AFTER INTRATRA-
CHEAL INJECTION OF TOXIC AND NONTOXIC
FOREIGN SUBSTANCES

IRA S. ROSS, M.D.

DURHAM, N. C.

The lining of the pulmonary alveoli and the mononuclear cells frequently seen in the alveolar spaces have been the object of numerous investigations. Recently several historical studies and reviews have been published (Miller ¹; Foot ²; Cappell ³; Josselyn ⁴; Fried ⁵) to which the reader is referred for a more extensive bibliography. In discussing the points at issue, only those considered to be current (Macklin ⁶) will be treated.

LITERATURE

There are three prevalent conceptions concerning the alveolar lining. The first is that there exists a complete endodermal epithelium continuous with that which lines the other respiratory passages. Recent supporters of this point of view are Miller,¹ Foot,² Gardner and Smith,⁷ Young,⁸ Parker and Weiss,⁹ El-Gazayerli,¹⁰ Bensley and Bensley,¹¹ Bensley and Groff ¹² and Bremer.¹³ The second contends that the alve-

From the departments of pathology and anatomy, Duke University School of Medicine.

1. Miller, W. S.: *The Lung*, Springfield, Ill., Charles C. Thomas, Publisher, 1937; in Cowdry, E. V.: *Special Cytology*, New York, Paul B. Hoeber, Inc., 1928, p. 71.

2. Foot, N. C.: *Am. J. Path.* **3**:413, 1927.

3. Cappell, D. F.: *J. Path. & Bact.* **32**:625, 1929.

4. Josselyn, L. E.: *Anat. Rec.* **62**:147, 1935.

5. Fried, B. M.: *Arch. Path.* **17**:76, 1934.

6. Macklin, C. C.: *J. Thoracic Surg.* **6**:82, 1936.

7. Gardner, L. U., and Smith, D. T.: *Am. J. Path.* **3**:445, 1927.

8. Young, J. S.: (a) *J. Path. & Bact.* **31**:265 and (b) 705, 1928; (c) **33**:363, 1930.

9. Parker, F., Jr., and Weiss, S.: *Am. J. Path.* **12**:573, 1936.

10. El-Gazayerli, M.: *J. Path. & Bact.* **43**: 537, 1936.

11. Bensley, R. D., and Bensley, S. H.: *Anat. Rec.* **64**:41, 1935.

12. Bensley, S. H., and Groff, M. B.: *Anat. Rec.* **64**:27, 1935.

13. Bremer, J. L.: *Contrib. Embryol.* (no. 83) **25**:83, 1935.

olar wall is incompletely covered by surviving endodermal cells. This has been maintained of late by Palmer,¹⁴ Wenslaw¹⁵ and Brodersen.¹⁶ The third holds that the wall is completely divested of its original epithelium and is composed only of cells of mesodermal origin (Josselyn¹; Loosli¹⁷; Fried¹⁸).

As for the alveolar phagocyte (dust cell, *Hertzfehlensell*, and so on), current authorities on this may be divided arbitrarily into three schools. First are those investigators who believe that this cell may arise from the pulmonary epithelium. Among them Cappell³ and Carleton¹⁹ have done recent work. Second is the school which holds it to be of mesodermal origin (Miller¹; Foot²; Gardner and Smith⁷; El-Gazayerli¹⁰; Josselyn¹; Loosli¹⁷; Fried¹⁸; Bloom²⁰; Wright²¹; Ungar and Wilson²²). The third school may be disregarded. To my knowledge, no one who in the last ten years has worked primarily to investigate this cell now believes the alveolar phagocyte to be derived from endothelium.

The different methods which have been applied to the study of these problems are reviewed here and criticized briefly in order to introduce the rationale which lies behind the present work.

In general, the methods employed may be listed as follows: (1) The outlining of the epithelial cells by specific staining; (2) the employment of vital and supravital technic; (3) embryologic and phylogenetic studies; (4) the treatment of basement membranes with stains for connective tissue to differentiate cell layers; (5) the alteration of the architecture of normal lungs in an endeavor to make epithelium more visible, and (6) the production of proliferation of the constituent cells.

In the first group, Bensley and Bensley¹¹ used intravenous gold sodium thiosulfate to demonstrate ground substance, silver stains by intratracheal injection to outline epithelial cells and Bielschowsky stains for reticular fibers. They concluded that the pulmonary alveoli of adult mammals are lined by a continuous cellular membrane. On the other hand, Josselyn¹ applied similar methods and came to the conclusion that

14. Palmer, D. M.: *Am. J. Anat.* **58**:59, 1936.

15. Wenslaw, A.: *Compt. rend. Soc. de biol.* **104**:611, 1930.

16. Brodersen, J.: *Ztschr. f. mikr.-anat. Forsch.* **32**:73, 1933.

17. Loosli, C. G.: *Anat. Rec.* **62**:381, 1935.

18. (a) Fried, B. M.: *Arch. Path.* **18**:865, 1934. (b) Fried, B. M., and Whitaker, L. R.: *Arch. Int. Med.* **40**:726, 1927. (c) Fried, B. M.: *Arch. Path.* **3**:751, 1927; footnote 5.

19. Carleton, H. M.: *Proc. Roy. Soc., London, s.B* **114**:513, 1934.

20. Bloom, W.: *Arch. Path.* **3**:608, 1927.

21. Wright, R. D.: *Am. J. Path.* **11**:497, 1935.

22. Ungar, J., Jr., and Wilson, G. R.: *Am. J. Path.* **11**:681, 1935.

the black lines seen on alveolar septums in silver nitrate impregnations of lungs may not be the boundaries of living cells. Moreover, he maintained that the alveolar wall is lined by a homogeneous ground substance on which the continuity of the cellular membrane depends.

Among the embryologists, Bensley and Groff¹² worked with macerated lungs of young rats at various stages from 20 day embryos to adults. They described the cuboidal alveolar epithelium as persisting until the twentieth day of gestation. After that time it became stretched into a thin membrane with successive inspirations just before and after birth, remaining thereafter as a distinct membrane demonstrable in adult life. Wenslaw¹⁵ studied late stages of embryonic life in man. He, in turn, claimed that in some places the pulmonary epithelium acquires an apparent discontinuity at the end of fetal life but that under certain conditions it reverts to the primitive condition of a continuous layer of cells loaded with lipid and lining the alveoli.

Gardner and Smith⁷ studied normal guinea pig lungs supravitaly stained with neutral red. They concluded that the alveolar epithelium and the local vascular endothelium play no part in the origin of the alveolar phagocyte. On the other hand, Cappell³ maintained that those cells reacting to particulate dyes introduced intratracheally are derived from cells in the alveolar lining and confine their activities to those spaces bounded by pulmonary epithelium.

By a combination of the fourth and fifth technics, Loosli¹⁷ studied lungs after collapse by phrenicotomy and pneumothorax and ligation of the pulmonary veins. He used the Mallory-Azan technic to stain ground substance and contended that: "At present there is no morphologic or histologic method which can demonstrate that the nucleated cells in the capillary meshes in the alveolar septa form a continuous histologic epithelium lining the spaces in the respiratory portion of the collapsed or expanded rabbit's lungs."

Parker and Weiss⁹ studied lungs altered by the changes occurring in mitral stenosis, using the Lee-Brown modification of Mallory's stain for connective tissue. In this manner they combined the fourth with the sixth approach and concluded that the alveolar wall is covered by flattened epithelial cells.

The approach which aims to induce cellular proliferation has, in turn, resulted in conclusions at variance with one another. Miller¹ studied desquamation as it occurs in lobar pneumonia. Foot² worked with supravitaly stained films and paraffin sections of supravitaly stained lungs in which milk pneumonia had been induced. Young⁸ used irritants introduced intrapleurally. El-Gazayerli¹⁰ added cytologic specific staining to Young's approach and described del Rio Hortega's silver carbonate stain as being specific for phagocytes. Sprunt, Martin

and Williams,²³ following up work done by Winternitz and his associates²⁴ on reactions in the lungs in which alveolar epithelial proliferation plays an important role, used bacteria and bacterial toxins. All these investigators were convinced of the integrity of the alveolar epithelial lining. On the other hand, Fried¹⁸ worked with vital stains and foreign bodies of a nontoxic nature introduced intratracheally. He expressed the opinion that there is no endodermal epithelial lining to the alveolus but that the large mononuclear cells found in the pulmonary alveoli in inflammatory and congestive processes are macrophages, originating from cells of mesenchymal origin found along the walls of the air vesicles.

It seems that much of the confusion in the past has been due to the fact that until very recently little attention has been paid to a correlation between the type of reaction and the strength of stimulus used, or to the time element concerned in the various inflammatory reactions in the lungs. Thus Fried,^{15b} using a nontoxic foreign substance (iodized poppyseed oil 40 per cent), brought out macrophages²⁵ with very little epithelial proliferation. Again, Cappell³ killed his animals only one-half hour after injecting one dye intravenously and another intratracheally. The intratracheal dye remained in cells on one side of the alveolar wall and the intravenous dye was seen only in cells on the other side. Thereupon he concluded that the alveolar phagocytes arise from the cells of the alveolar lining and do not enter the underlying tissues.

Closer attention has recently been given to the time factor in studies of inflammatory processes in the lungs. Young^{8c} found that the visible cycle of changes in the pulmonary alveolar epithelium of the rabbit covers a period of eight days after the initial stimulus has been administered, waxing to cellular proliferation on the third or fourth day and thereafter regressing. Moreover, Sprunt, Martin and Williams²³ noted that on the third or fourth day after intratracheal administration of staphylococcus toxin, when the polymorphonuclear cell reaction had begun to subside, the walls of the alveoli and the tissues surrounding the bronchi and bronchioles showed an infiltrate of lymphocytes and monocytes. They also pointed out that, in addition to the presence of these cells, the walls of the alveoli became definitely thickened with cuboidal cells which had vesicular nuclei and scanty, pale-staining cytoplasm. These cells were believed to be epithelial, but it was not possible

23. Sprunt, D. H.; Martin, D. S., and Williams, J. E.: *J. Exper. Med.* **62**: 73 and 449, 1935.

24. Winternitz, M. C.; Smith, G. H., and McNamara, F. P.: *J. Exper. Med.* **32**:199, 205 and 211, 1920.

25. In the following pages all cells of the mononuclear phagocytic series will be referred to as macrophages.

to determine their exact nature because of limitations imposed by the hematoxylin and eosin-stained material.

The present study was undertaken to see whether vital and supravital staining of the lungs in such a series of animals would aid in distinguishing between these two types of cells. Furthermore, it was thought that a comparative study of the reactions in the lungs to a substance of toxic nature and to one of a nontoxic nature might be of value. Each of the substances used to induce cellular proliferation had led its respective employer in the past to contradictory conclusions on the nature of the alveolar wall. To determine, if possible, what it was that caused this divergence of opinion and thereby to throw further light on the nature of the cells in question, the following experiments were undertaken.

METHODS AND MATERIALS

Twenty-one young adult rabbits, weighing between 2 and 4 Kg., were used in this experiment. The presence of *Bacterium leptisepticum* and *Bacillus bronchisepticus* in the nares was ruled out by means of cultures after silver nitrate instillations, as suggested by Meyer.²⁶

Toxin.—Toxin was made from the hemolytic strain of *Staphylococcus aureus* employed by Rigdon and his associates²⁷ and was prepared by the method described by Parker, Hopkins and Gunther.²⁸ The content of one vaccine bottle of toxin was employed throughout all these experiments.

Iodized Oil.—The iodized oil used was poppyseed oil 40 per cent (lipiodol-Lafay N.N.R.).

Inoculation.—The technic described by Sprunt, Martin and Williams²³ was employed for intratracheal inoculation.

Dose.—Preliminary experiments showed that 0.05 cc. of the toxin per animal produced the optimal proliferative type of lesion, while large doses produced a hemorrhagic and necrotizing lesion and in some cases, death within two or three days. The toxin was always made up to 1 cc. with physiologic solution of sodium chloride.

Iodized oil was administered in doses of 1 cc. per kilogram, an amount which was considered optimal by Fried^{18b} in working with cats.

Necropsy.—With the exception of 2 rabbits stained by intravascular perfusion, each animal was killed by a blow at the base of the skull, and necropsy was performed at once. The gross changes were not significant, so only the microscopic ones are given.

Fixation and Staining.—The lungs of 2 animals were stained with neutral red according to Hu's²⁹ modification of Forkner's³⁰ technic. Those of 16 other

26. Meyer, K. F., in Jordan, E. O., and Falk, L. S.: *The Newer Knowledge of Bacteriology and Immunology*, Chicago, University of Chicago Press, 1928, p. 607.

27. Rigdon, R. H.; Joyner, A. L., and Ricketts, E. T.: *Am. J. Path.* **10**: 425, 1934.

28. Parker, J. T.; Hopkins, J. G., and Gunther, A.: *Proc. Soc. Exper. Biol. & Med.* **23**:344, 1926.

29. Hu, C. H.: *Proc. Soc. Exper. Biol. & Med.* **29**:258, 1931.

30. Forkner, C. E.: *J. Exper. Med.* **52**:379, 1930.

animals were removed immediately after death and immersed in a beaker of Forkner's staining solution. This was maintained at 37 C. in a water bath, and the lungs were gently perfused for fifteen minutes with the same solution introduced through the trachea. The inflow of staining solution was regulated so as not to overdilate the lungs and was followed by fixation after the method described by Hu.²⁰ Dehydration and clearing were carried out as described by Hu²⁰ in all cases except one. In this case the technic described by Gardner³¹ was used.

In the case of the lungs stained with neutral red, three sections from each block were prepared for study. One of these was stained with hematoxylin and eosin. This usually resulted in washing out all the neutral red. The second was stained for nuclear detail by the technic described by Gardner,³¹ using Harris' hematoxylin without acetic acid. The third was studied stained only with neutral red after dissolving excess mercuric chloride crystals in aqueous solution of iodine. All observations with low magnification were best made after staining with hematoxylin and eosin. The sections stained using Gardner's technic allowed comparisons of nuclear structure to be made between sections stained with neutral red alone and those stained with hematoxylin and eosin. The plain neutral red sections made possible a better discrimination between cells which were alive and cells which were dead at the time the animal was killed.

The lungs of animals which received carmine and trypan blue were fixed by gentle intratracheal perfusion with 10 per cent neutral solution of formaldehyde U. S. P. Sections were stained with brazilin for study under high magnification, and others with hematoxylin and eosin.

EXPERIMENTAL OBSERVATIONS

EXPERIMENT 1.—This experiment was designed to allow a study of changes in the lungs after administration of optimal doses of toxic and nontoxic substances.

Fourteen rabbits were used. Six received 0.05 cc. each of staphylococcus toxin intratracheally. These were killed after thirty-two, forty-eight, seventy-two, ninety-six hours and eight days, respectively. The ninety-six hour experiment was represented by 2 animals. The other 8 animals received 1 cc. each of iodized oil per kilogram and were killed at the same intervals, the ninety-six hour experiment being represented by 4 animals. The lungs of these animals were stained with neutral red and sections prepared for study as described in the foregoing section.

Changes Produced with 0.05 Cc. of Toxin.—After thirty-two hours the reaction to toxin was characterized by rather copious exudation of cells into the terminal air spaces about the larger bronchi, as shown in figure 1, *J*. The majority of these cells were active macrophages. A few degenerating macrophages and polymorphonuclear cells were present, but both were being phagocytosed. Small amounts of serum and fibrin were seen in the alveoli. Slight thickening of the alveolar septums was observed. This was occasioned by moderate edematous swelling and by increase of cells within the septums. The bronchial epithelium appeared to be intact.

As in the lungs undisturbed by pathologic change, the alveolar walls for the most part seemed to have no distinct cellular lining. In many places the capillaries seemed to be exposed to the alveolar air. In others, flattened cells with darkly stained oval nuclei seemed to have separated from the surface of the alveoli.

31. Gardner, L. U.: Proc. Soc. Exper. Biol. & Med. **24**:646, 1927.



Fig. 1.—1, thirty-six hours after intratracheal injection of 0.05 cc. staphylococcus toxin. 2, thirty-six hours after intratracheal injection of 1 cc. per kilogram of iodized oil. 3, forty-eight hours after intratracheal injection of 0.05 cc. of staphylococcus toxin. 4, forty-eight hours after intratracheal injection of 1 cc. per kilogram of iodized oil. Hematoxylin and eosin; $\times 85$.

In a few places a group of fibroblasts projected into the alveolar space. Rarely such a group was seen to project on both sides of one septum so that their connecting protoplasmic bands apparently passed through the septal wall.

After two days areas of heavy consolidation about the bronchi were seen, and the alveoli in these areas were filled with a serous exudate containing great numbers of leukocytes. When this reaction was most severe, exudation of leukocytes and of red cells was so great as to obscure the pattern of the lung. The white cells of the exudate were chiefly degenerated macrophages and polymorphonuclear cells, although numerous large active macrophages were present. The bronchial epithelium showed considerable degenerative change and in places was desquamated in sheets.

The edematous swelling, cellular infiltration and vascular engorgement of the walls of the alveoli, coupled with the intense serous and cellular exudate into the alveoli themselves, made definition of a lining membrane impossible within the areas of intense inflammatory reaction at this stage.

After three days the microscopic appearance (fig. 2, 5) was still one of rather heavy consolidation about the large bronchi. Evidences of destruction and of proliferation were seen in the bronchial epithelium, and in places there were masses of red and white cells caught in strands of fibrin within the bronchi. In patches within areas of consolidation, retraction of the exudate from the alveolar walls permitted the architectural pattern of the lungs to be seen. Once again the large, active macrophages had come into prominence, although they were still outnumbered by degenerated cells. Numerous giant cells were present. Only in those places where no retraction had occurred were there great numbers of red cells and polymorphonuclear cells within the alveoli. An explanation of the rapid removal of these degenerating cells, along with the remainder of the previous day's alveolar exudate, could be seen in the longitudinal section of the termination of a bronchiole. This could be followed for a considerable distance, starting at its ramifications among alveoli, which showed some evidence of resolution. Near its terminal branches the predominant cells were active large macrophages. As observation proceeded proximally, the character of the exudate changed and became more nearly like that seen in the alveoli on the previous day. The nuclei of the macrophages became pyknotic, the polymorphonuclear cells were largely disintegrated, and the red blood cells were represented, for the most part, by a homogeneous matrix of orange pigment.

In those areas in which the exudate had separated from the septum, a change in the alveolar lining was evident. In these places there was a definite cellular border on the inner surfaces of the alveoli. The constituents of this border were very low cuboid cells, generally with round reticular nuclei which had one or two nucleoli and a rather heavy nuclear membrane. Occasionally the nuclei were somewhat oval, with their long diameter parallel to the surface of the alveolar wall. These cells were seen to be contiguous, the distance between adjacent nuclei being usually about one third of the diameter of the nucleus itself. It was not difficult to trace this cellular alveolar lining from the alveoli and terminal ramifications of the air ways to the walls of the bronchioles, where it gradually assumed a more compact cuboidal arrangement; then, to the columnar epithelium of the bronchioles and bronchi. It was very difficult to distinguish the cells lining the alveoli from the large macrophages of the exudate on the basis of cytologic characteristics alone when stained with hematoxylin and eosin. In regions of epithelial proliferation the septums were very greatly thickened, the capillaries within them were dilated and the proliferation of fibroblasts between the rows of cuboid cells was still evident.



Fig. 2.—5, seventy-two hours after intratracheal injection of 0.05 cc. of staphylococcus toxin. 6, seventy-two hours after intratracheal injection of 1 cc. per kilogram of iodized oil. 7, ninety-six hours after intratracheal injection of 0.05 cc. staphylococcus toxin; inset shows proliferated alveolar epithelium. 8, ninety-six hours after intratracheal injection of 1 cc. per kilogram of iodized oil; inset shows macrophages with clear cytoplasm. Hematoxylin and eosin. The insets in 7 and 8 show magnification $\times 173.3$; the parts otherwise show magnification $\times 86.7$.

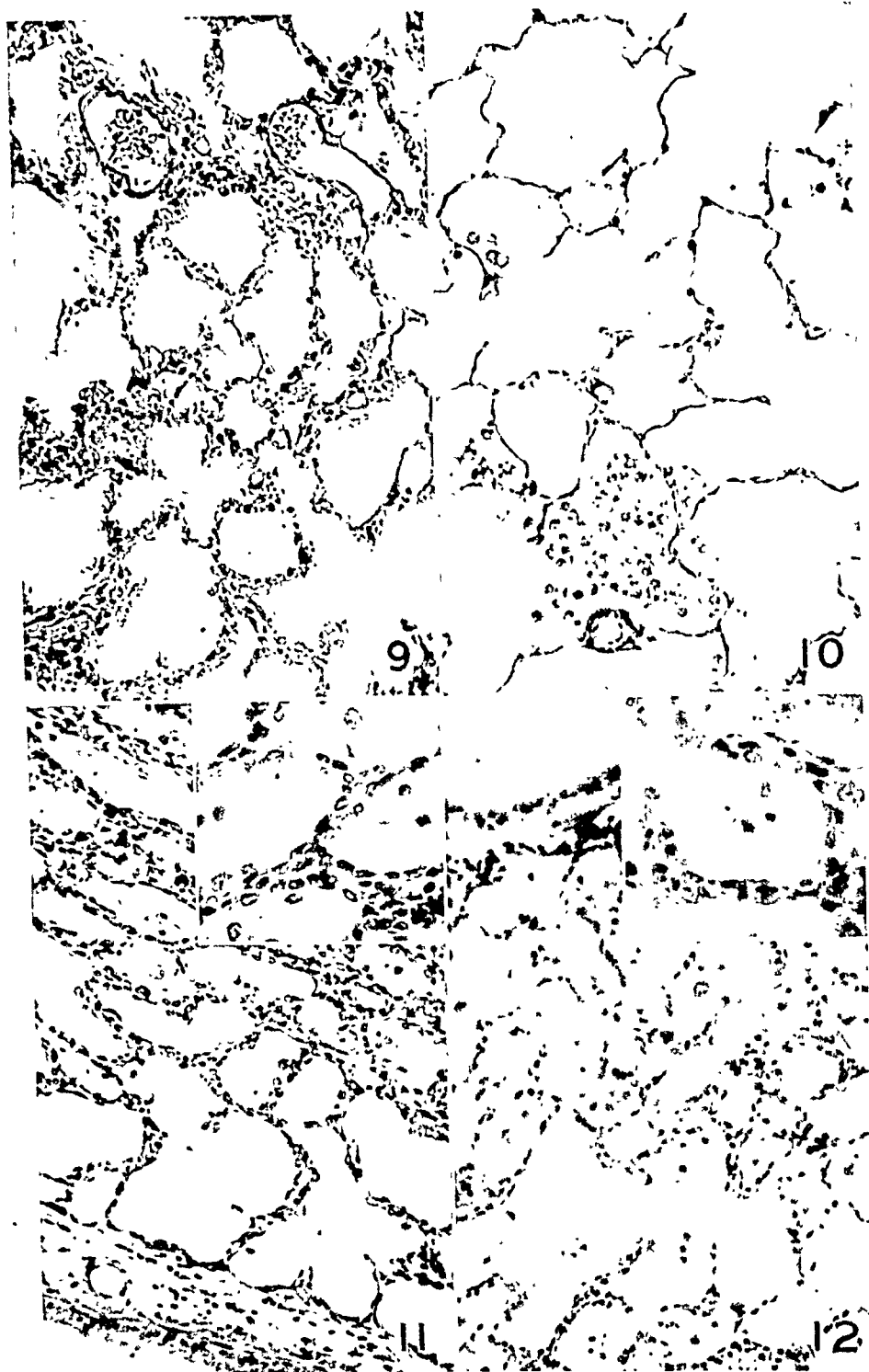


Fig. 3.—9, eight days after the intratracheal injection of 0.05 cc. of staphylococcus toxin. 10, eight days after the intratracheal injection of 1 cc. per kilogram of iodized oil. 11, ninety-six hours after intratracheal injection of 0.03 cc. of staphylococcus toxin; inset shows proliferated epithelium. 12, ninety-six hours after the intratracheal injection of 0.01 cc. of staphylococcus toxin. Compare inset with insets 7 and 11. Hematoxylin and eosin. The insets show magnification $\times 180$; otherwise the parts show magnification $\times 86.7$.

After four days the general microscopic appearance (fig. 2,7) was still one of dense consolidation about the larger bronchi. Desquamation and proliferation of the bronchial epithelium were still evident. The exudate within the bronchi as compared with that of the previous day seemed to be chiefly of a serous or mucinous nature. The few cells seen within it were no longer a conglomeration of degenerated red and white cells but fairly large macrophages and giant cells (in fig. 4, 16 and 17) such as were seen in those alveoli of the previous day which had begun to show evidences of resolution. The vast majority of the cells of the alveolar exudate were not active macrophages. The alveolar septums were still greatly swollen. However, the most striking and characteristic feature of the picture presented by the lungs at this stage was the distinct layer of cuboid cells lining the alveoli in places where the process of resolution was in progress. Compared with those of the previous day, more alveoli displayed this lining, and its cells were more definitely cuboid. It was evident that in places that exudate had undergone fibrous organization and had, in turn, become epithelialized. In consequence, some of the terminal air spaces showed both the septal wall and the exudate within covered by an epithelial pavement. Continuity from bronchial to alveolar epithelium was demonstrable here, although the sections were not cut as fortunately as were those of the previous day.

In the neutral red sections the cells of the exudate were seen to be typically stained macrophages, the cytoplasm of which was loaded with brilliant orange and brick red vacuoles of various sizes. The smaller cells seemed to contain the finer vacuoles whereas in the larger some of the vacuoles attained a size nearly equal to half the diameter of the nucleus. Around alveoli which contained 5 to 10 such well stained macrophages the cuboid epithelial cells were seen to contain no dye or only a few specks of it (fig. 4,15). This contrast was clear and distinct. The neutral red was given this animal by intravascular perfusion, so that the stain must have passed through the epithelial border to gain access to the phagocytes inside the alveoli. It is to be noted that where the staining was intense the tall columnar cells of the bronchial epithelium contained very fine dark droplets of neutral red.

By the eighth day a large amount of resolution had occurred (fig. 3,9). The exudate had almost entirely disappeared from the alveoli. No longer was there any evidence of epithelial proliferation. The only record of the preceding disturbance was that manifest in the interstitial tissues of the septums. These were still considerably thickened by an abundance of what proved to be collagenous tissue when stains for connective tissue were applied.

Changes Produced with Iodized Oil.—After thirty-two hours there were large numbers of macrophages with vacuolated cytoplasm within alveoli and smaller bronchioles (fig. 1,2). These macrophages had taken up neutral red in vacuoles of various sizes. However, it seemed that the more large clear vacuoles a cell contained, i. e., the more iodized oil it must have ingested, the fewer were the vacuoles of neutral red. In a few places the alveolar septums were slightly thickened by very mild proliferation of fibroblasts.

The flat cells with oval nuclei thought to line the alveoli had taken up no neutral red. Macrophages were frequently seen projecting from the alveolar walls, but these did not resemble an epithelium.

After forty-eight hours there was little change in the microscopic picture (fig. 1,4) beyond that described in the foregoing paragraph. There was a slight increase in the number of vacuolated macrophages in the exudate, and once again there seemed to be a definite connection between the amount of iodized oil and the amount of neutral red phagocytosed by the cells. There was a slight

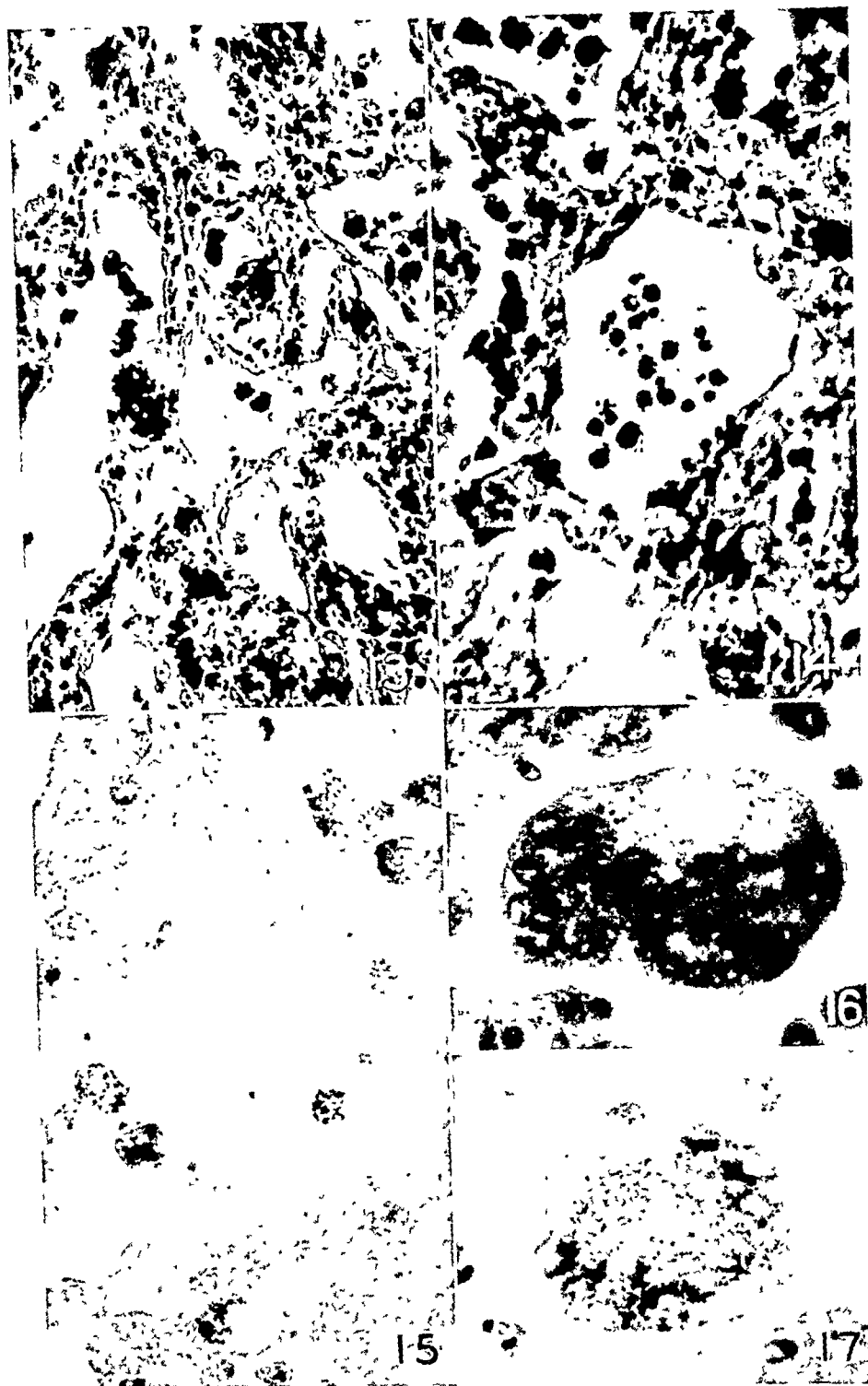


Fig. 4.—13, seventy-two hours after intratracheal injection of 0.05 cc. of staphylococcus toxin (carmine injected intravenously). Brazilian counterstain; $\times 173.3$. 14, same as 13. 15, ninety-six hours after intratracheal injection of 0.05 cc. of staphylococcus toxin. Neutral red and hematoxylin; $\times 390$. 16, ninety-six hours after intratracheal injection of 0.05 cc of staphylococcus toxin. Hematoxylin and eosin; $\times 390$. 17, same as 16, stained with neutral red and hematoxylin; $\times 390$.

increase in the septal thickening produced by interstitial proliferation of fibroblasts. There was a slight serous exudate in some of the alveoli.

In a very few places where cells lining the alveoli appeared to be present in continuous flattened sheets, there was indication of epithelial proliferation. These cells contained no neutral red. Macrophages in small aggregations were seen in the loose connective tissues.

At the end of seventy-two hours the general microscopic appearance had been altered very little (fig. 2, 6). There seemed to be a few more cells in the alveolar exudate, including a few polymorphonuclear cells. Practically none of the serous exudate was left within the alveoli. The changes in the septums were about the same as they were on the previous day. Where epithelial proliferation had occurred, it was very slight. The bronchial epithelium remained intact.

The cells of the exudate, although still practically all macrophages, had changed somewhat in appearance. The large clear vacuoles had in general become smaller and were more numerous within the individual cells. The reciprocal relationship between the number of clear cytoplasmic vacuoles and those filled with neutral red was again evident. Moreover, there was a new order of smaller macrophages which contained no clear cytoplasmic vacuoles but which had taken up neutral red in small dark vacuoles arranged in a rosette at the base of the nucleus. Macrophages in the loose connective tissues were more in evidence than on the previous day.

There was little difference between the picture presented at ninety-six hours (fig. 2, 8) and those of the previous three days. Rare instances of alveolar epithelial proliferation were seen in which cuboid cells were arranged in continuous sheets along the alveolar walls. These cells contained no vacuoles of neutral red. Comparatively few of the macrophages in the alveoli contained large clear cytoplasmic vacuoles. More of them contained fairly numerous neutral red vacuoles of varying sizes and shapes. The polymorphonuclear cells gave evidence of degeneration and many of them had been ingested by macrophages.

After eight days the reaction had subsided to a marked degree, as shown in figure 3, 10. A great majority of the alveoli were entirely clear, although occasional air spaces were seen to contain rather large aggregations of macrophages and debris. In a few of these, epithelial proliferation was evident. More alveoli contained 1 or 2 free macrophages than is the case in sections of normal lungs, but only a few of these scattered macrophages showed vacuoles containing iodized oil, whereas the majority in the large aggregations did possess them. Most of the macrophages gave evidence of having undergone some degree of degeneration. Their cytoplasm appeared frayed and their nuclei pyknotic. Most of them contained neutral red in only a few, fairly large vacuoles. They seemed to be older cells which had lost a large measure of their phagocytic power.

EXPERIMENT 2.—This experiment was designed to show whether the differences in result between toxin and iodized oil were due to a qualitative difference in the toxin or merely to a quantitative difference in the toxicity of the two substances.

Of the 4 rabbits used in this experiment 2 were given 0.03 cc. each of toxin and 2 received 0.01 cc. each of toxin. All the animals were killed four days after the injections.

Changes Produced with 0.03 Cc. and with 0.01 Cc. of Toxin.—Four days after the administration of 0.03 cc. of toxin the reaction (fig. 3, 11) was by no means as intense as that seen after 0.05 cc. had been used. The bronchial epithelium was intact, and the alveoli were not choked with the copious exudate seen after

the larger dose. The most striking similarity was in the epithelial proliferation. Because the exudate was generally so slight, the epithelial cells stood out very clearly. Everywhere within the areas of reaction, on both sides of the alveolar septums sheets of low cuboid cells with slightly oval nuclei were seen. The epithelial border thus produced was seen to be continuous all the way around the alveoli, and where the sections had been cut opportunely, it was possible to trace the continuity of epithelium from the bronchi and bronchioles through what must have been the alveolar ductules and the alveolar atria and saccules into the alveoli of the lungs. These cells remained for the most part unstained by neutral red. At times very fine specks of the dye were seen near the nuclei. The macrophages of the exudate in contrast to those seen after administration of the larger dose of toxin had not taken up the stain with great avidity. Their cytoplasmic vacuoles, although of fair size, were comparatively few. A great many of these cells contained no vacuoles at all, but their nuclei had taken up the stain. Again the septums were thickened by proliferation of fibroblasts, among which were seen a few macrophages.

It would be a very difficult matter to distinguish the reaction to 0.01 cc. of this staphylococcus toxin (fig. 3, 12) from that incited by the administration of 1 cc. of iodized oil per kilogram. Practically the only distinguishing feature was that the macrophages in the exudate did not contain the large clear cytoplasmic vacuoles. The bronchial epithelium appeared to be intact. There was no consolidation, and the cells in the exudate were practically all large macrophages. They appeared to be undergoing varying degrees of cytolysis and contained even less neutral red than in the preceding case. In many places they were seen to have been ingested by cells of their own species. The few giant cells present contained only 2 or 3 nuclei. Where septal thickening existed, it was occasioned by moderate proliferation of fibroblasts. Only in areas where the reaction was strongest had there occurred slight epithelial proliferation.

EXPERIMENT 3.—The third experiment was undertaken for only one purpose. This was to investigate whether or not macrophages of the exudate were capable of wandering from the connective tissue into the alveoli and back again, for it might be contended that they were only specialized epithelial cells.

Three rabbits were used. Each received 1 cc. of 1 per cent trypan blue intravenously for three days before receiving 0.05 cc. of toxin intratracheally. Forty minutes after the toxin was introduced 3 cc. of 1 per cent carmine was put into the trachea of one of these animals. The forty minute delay was interpolated to forestall adsorption of the toxin by carmine. On the day of inoculation another animal received 1 cc. of 1 per cent carmine intravenously and 1 cc. by the same route on each of two successive days before death. The third animal continued to receive daily intravenous injections of 1 cc. of 1 per cent trypan blue. All 3 were killed at seventy-two hours.

Distribution of Vital Dyes.—The reaction was similar to that found in the rabbits which were killed three days after receiving 0.05 cc. of the toxin. The changes are shown in figure 4, 13 and 14. Macrophages containing carmine or trypan blue or both were seen in the alveoli and also in the interstitial tissue.

SUMMARY OF ANATOMIC CHANGES

In brief, the reactions in the lungs which formed the basis of this study took place as follows: First there was moderate exudation of polymorphonuclear cells. This was rapidly followed by outpouring of

large numbers of macrophages, which ingested the polymorphonuclear cells. Within the first thirty-two hours there occurred moderate proliferation of fibroblasts within the septal walls, and degenerative changes became evident in the pulmonary epithelium. These changes were made manifest by a mild amount of epithelial desquamation. Where this occurred, fibroblasts were sometimes seen to grow from septal interstices into the alveoli, and when they projected from both sides of one septum the appearance was that of alveolar pores (Miller;^{1a} Macklin;³² Loosli³³). During the second day the epithelial damage became further evident. Serum and red cells seeped out into the alveoli, and there was further outpouring of macrophages and polymorphonuclear cells. By this time the proliferation of macrophages within the connective tissues of the lungs themselves became strikingly evident. Multinucleated giant cells were plentiful in the exudate. The bronchial epithelium began to show degenerative changes, and in many places it was desquamated in sheets. By the third day the reaction began to resolve. A large amount of exudate was apparently ejected via the larger respiratory passages. The macrophages attained their greatest numbers and were seen to ingest residual debris. Proliferation of alveolar epithelium became evident along the septal borders, and in places epithelialization of the exudate occurred. By the fourth day this epithelial proliferation reached its height. Sections of vitally stained tissue showed these cells to be non-phagocytic and distinct from the macrophages present. Moreover, their continuity with the cells lining the bronchioles and bronchi could be traced. Quite a few small round cells, judged to be degenerated macrophages, were seen in the exudate. After eight days the only evidence of the damage sustained by the tissues was a moderate thickening of septal walls occasioned by proliferation of fibroblasts. Even these appeared to be shrunken, as judged by their nuclei and by the amount of collagenous tissue present.

After the introduction of 1 cc. of iodized oil per kilogram into the trachea, no such severe inflammatory reaction occurred. It was obvious that little, if any, damage was sustained by the pulmonary epithelium. If an initial polymorphonuclear cell reaction occurred, it was missed in this study. By the end of the first thirty-two hours large numbers of macrophages were seen within the alveoli. They seemed to have taken up large quantities of the iodized oil, as evidenced by their colorless cytoplasmic vacuoles. By the end of the third day many of the original macrophages seemed to have undergone degenerative changes. At this time many younger macrophages and a few polymorphonuclear cells were present in the exudate. The former were seen to ingest cells of their

32. Macklin, C. C.: *J. Anat.* **69**:188, 1935; *Arch. Path.* **21**:202, 1936.

33. Loosli, C. G.: *Arch. Path.* **24**:743, 1937.

own species, as well as degenerated polymorphonuclear cells. Small aggregations of macrophages within the connective tissues about blood vessels and bronchi of the lungs were seen. The only other interstitial reaction was a very slight proliferation of fibroblasts within the walls of a few of the septums. Very rarely did epithelial proliferation occur. By the eighth day the majority of the remaining macrophages seemed to be aggregated in isolated air spaces.

That the difference between these two reactions was one of degree and not specific was illustrated by the titration series. This, moreover, corroborated the work done by Young.^{8c} With 0.03 cc. of staphylococcus toxin, epithelial proliferation reached as high a peak as it did with the stronger dose. On the other hand, in the animals which received only 0.01 cc. each this proliferation was little more in evidence than in the series given iodized oil. Thus the results showed that wherever the stimulus was sufficiently strong and acted for a brief period of time the epithelial response went through a cycle lasting about eight days, waxing to the height of proliferation by the third or fourth day and thereafter regressing.

COMMENT

It should be emphasized again that for practical purposes alveolar epithelium is not visible in ordinary histologic preparations of normal tissue. However, when stimulated with sufficient intensity, it proliferated within three or four days, appearing as a lining of cuboid cells. These were not seen singly or in groups of 3 or 4, but formed a continuous layer completely surrounding the alveoli and bordering both sides of the septums. They were seen at that time to be continuous with the lining of the bronchioles and bronchi. When the alveolar epithelium could be seen as an entity, it was apparent that its constituent cells were not phagocytic (fig. 4, 15). El-Gazayerli¹⁰ demonstrated this well. His work has been corroborated in the present study.

It has been suggested by some workers that what appears to be a lining of low cuboidal epithelium is merely a flattened layer of mesothelial macrophages lined up on the alveolar wall in a resting state. Others have contended that the cells lining the alveolar wall, although of epithelial nature, are themselves phagocytic. During the course of this study typically stained macrophages, loaded with vacuoles containing neutral red or containing an abundance of carmine or trypan blue, were seen to protrude from the alveolar wall. These occurred singly, in groups of 3 or 4 or in rounded bunches. Where no proliferation of alveolar epithelium was evident, these cells might well have been interpreted as forming the alveolar lining. Cappell,³ apparently considering them to be alveolar epithelial cells, went further in his observations and concluded that these phagocytes do not move outside of the alveolar

epithelium. He arrived at this conclusion by introducing one vital stain into the trachea and another of a different color into the blood stream, killing his animals at the end of one-half hour. The intratracheal dye was seen in phagocytes within the alveoli. The intravenous dye was seen in phagocytes in the interstitial tissues. He concluded that two phagocytic systems were at work. The present study has shown that dyes introduced intravenously and intratracheally may become well mixed on either side of the epithelial border, given sufficient time and conditions favoring activity of macrophages. It should not be assumed that return of macrophages from alveoli to the interstitial tissues is taken to be the usual course of events. On the contrary, it appears that the phagocytes usually are expectorated along with the foreign substance which they have ingested. This is the course taken by motile entities that find their way into alveoli, as has been so long demonstrated to be true for those parasites that migrate from the blood stream into the lungs and are then expectorated or swallowed. However, when the usual path of least resistance is impeded, as it might be when the alveoli are choked with an exudate, it appears that the macrophages may wander back along the path from which they came.

It is evident from the present study and from the work of others (Gardner and Smith;⁷ El-Gazayerli;¹⁰ Wright;²¹ Ungar and Wilson²²) that the macrophages do not arise from the alveolar epithelium. Foot at one time held that they might arise from the capillary endothelium of the lungs. In subsequent work he² became convinced that this is not the case and reversed his former stand. Nevertheless, in every section used in the present study a careful examination of the endothelium was made. In no instance was there any indication of transition from endothelial cells to macrophages. Maximow³⁴ described transformation of lymphocytes and monocytes into macrophages in tissue cultures. Forkner³⁵ worked with fixed tissues of rabbits vitally stained with neutral red. He described monocytes as being present in all stages of development in all the lymph nodes of the body except the large mesenteric group. With regard for previous work of this nature, in the present investigation a careful study was made of lymphoid accumulations and lymph nodes within the sections. Usually a few mononuclear phagocytic cells were found within each. However, it is to be emphasized that nowhere was there seen anything to suggest transition from lymphocytes to macrophages. As the result of studies of cells supravitaly stained with neutral red and janus green, Sabin, Doan and Cunningham³⁶ concluded that two types of mononuclear phagocytic

34. Maximow, A. A.: *Proc. Soc. Exper. Biol. & Med.* **24**:570, 1927.

35. Forkner, C. E.: *J. Exper. Med.* **52**:385, 1930.

36. Sabin, F. R.; Doan, C. A., and Cunningham, R. S.: *Contrib. Embryol.* (no. 82) **16**:125, 1925.

cells originate in the connective tissues. These they termed monocytes and clasmatocytes. In view of their work, in the present study close attention was paid to the cells in the connective tissues of the lungs. Especially in those sections where the reaction was of considerable intensity, numbers of macrophages were seen in the loose connective tissues about the larger bronchioles, bronchi and blood vessels, particularly the veins. However, so many of the cells were intermediate between the forms containing fine brick red vacuoles of neutral red situated in a rosette at the nuclear hof (Sabin's monocyte) and those larger cells the cytoplasm of which contained an abundance of neutral red vacuoles of various sizes, shades and tints distributed throughout the cytoplasm, with little or no suggestion of rosette arrangement (Sabin's clasmatocyte), that these two were considered morphologic variants of the same cell. This interpretation is consistent with Maximow's opinion concerning monocytes and falls in line with work done by Hetherington and Pierce,³⁷ who observed transformation of monocytes into macrophages (Sabin's clasmatocytes) and epithelioid cells in tissue cultures of buffy coat. Following Metchnikoff,³⁸ these cells have been referred to in this paper as macrophages. They are considered to be connective tissue wandering cells, and it should not be assumed that any specific locale is designated as being their site of origin.

The Langhans type of giant cells (fig. 4, 16 and 17) (Forkner³⁹) which were so frequently seen in the lungs after the introduction of staphylococcus toxin apparently did not arise in order that larger quantities of foreign substance might be handled. This opinion was supported by the presence of many such giant cells in the reactions to toxin, whereas they were very little in evidence after the introduction of iodized oil. It seemed obvious that the reaction occasioned by the introduction of iodized oil into the lungs was mainly concerned with removing a comparatively harmless foreign body, whereas in the case of toxin the reaction was a matter of inflammation and repair after damage done to the tissues by a toxic substance.

Finally, with regard to the two types of cell discussed in this paper, a word might be said as to the general function of each. It seems likely that the difference between complete resolution and scarring after inflammatory reactions in the lungs depends on the extent to which pulmonary epithelium is damaged or destroyed. When the strength of staphylococcus toxin is increased over that used here, it is customary to

37. Hetherington, D. C., and Pierce, E. J.: *Arch. f. exper. Zellforsch.* **12**:1, 1931.

38. Metchnikoff, É.: *Lectures on the Comparative Pathology of Inflammation*, translated by F. A. Starling and E. H. Starling, London, Kegan Paul, French, Trübner & Co., 1893.

39. Forkner, C. E.: *J. Exper. Med.* **52**:279, 1930.

see necrosis and subsequent scarring of the tissues. Robertson and Uhley⁴⁰ studied the cellular changes which occur in lobar pneumonia. This disease is attended with an infection of the blood stream, as a result of which the capillary walls must sustain severe toxic damage, which becomes manifest during the stage of red hepatization. Consequently the pathologic picture is even more complex than that seen in the material described in this paper. However, they noted that with the appearance of phagocytic macrophages the pneumococci decreased in numbers and that resolution of the pneumonic process followed. Thus the pulmonary epithelium is considered to maintain a surface and the macrophages to act as a defense against toxic and nontoxic foreign substances.

SUMMARY

In these experiments vital and supravital staining technics have been used in the study of cellular reactions consequent to the introduction of both nontoxic and toxic substances into the lungs of rabbits. It has been shown that two distinct types of cells were stimulated. One of them was a nonphagocytic cell lining the alveoli, similar to and continuous with the epithelial cells of the bronchioles. The other type of cell was the macrophage.

The first type arose from the epithelial cells which line the alveoli. It was stimulated slightly by nontoxic substances but was increased to an appreciable extent only when toxic material was injected.

The other type of cell, the macrophage, was stimulated both by the nontoxic and the toxic substances. It has been shown to arise from the connective tissue macrophage and to migrate freely across the alveolar epithelial borders.

40. Robertson, O. H., and Uhley, C. G.: *J. Clin. Investigation* **15**:115, 1936.

EXPERIMENTAL MUSCULAR DYSTROPHY IN THE GUINEA PIG

A NUTRITIONAL MYODEGENERATION

HERMAN CHOR, M.D.

AND

RALPH E. DOLKART, M.D.

CHICAGO

In previous studies we reported on the roles played by activity¹ and by the peripheral nerves² in the maintenance of the trophic state of skeletal muscle. It was shown that the mild changes which occur in atrophy of skeletal muscle due to simple disuse are distinctly different from the degenerative ones observed in atrophy following peripheral nerve section. The objective of the present study was to investigate the nutritional factors concerned in maintaining the normal structure and activity of skeletal muscle.

It is well known that inanition or long-continued fasting gives rise to structural changes in skeletal muscles. These changes have been considered "primary" and often occur before any obvious lesions can be observed in other organs. The histologic changes in the hibernating frog have been described by Kremer³ and those in the eel by d'Ancona.⁴ The latter described fading of the cross striations and of the Q disks and disappearance of sarcosomes. He held that these elements are chiefly substances used to yield energy during starvation. The M and Z lines and the sarcolemma were found to be resistant. Myers and Fine⁵ found that the pathologic changes occurring in rabbits starved for a long period of time are associated with changes in creatine content, an increased amount of creatine appearing in the urine.

Pathologic changes in skeletal muscle occur with certain vitamin deficiencies. Hyaline necrosis of muscle fibers has been observed in

From the Department of Nervous and Mental Diseases and the Department of Chemistry, Northwestern University Medical School.

This work was carried on with the assistance of a grant from the Council on Physical Therapy of the American Medical Association.

1. Chor, H., and Dolkart, R. E.: *Am. J. Physiol.* **117**:626, 1936.

2. Chor, H.; Dolkart, R. E., and Davenport, H. A.: *Am. J. Physiol.* **118**: 580, 1937.

3. Kremer, J.: *Virchows Arch. f. path. Anat.* **274**:729, 1929-1930.

4. d'Ancona, U.: *Am. J. Anat.* **39**:168, 1927.

5. Myers, V. C., and Fine, M. S.: *J. Biol. Chem.* **15**:283, 1913.

experimental scurvy in guinea pigs (Hojer ⁶ ; Meyer and McCormick ⁷ ; Dalldorf ⁸). Numerous workers have reported muscle lesions appearing in laboratory animals following administration of vitamin-deficient diets. Goettsch and Pappenheimer ⁹ reported on muscle lesions in guinea pigs and rabbits on a special prepared diet, complete except for vitamin E, described as progressive dystrophy of the voluntary muscles. Addition of vitamin E to the special diet, however, did not prevent development of the lesions. Hence the lack of this vitamin could not have been the sole factor responsible for the muscle changes. They ruled out such factors as starvation, infection and scurvy. Therefore, some unknown factor in their special diet was considered responsible for the muscular lesions. Morgulis and Spencer ¹⁰ repeated this work, using the Goettsch-Pappenheimer diet 13, and obtained similar results. They stated, however, that they were able to prevent as well as cure the "dystrophy" by feeding the following supplements along with the dystrophy-producing diet 13: fresh green alfalfa, lettuce and vitamin E (wheat germ oil), or dry alfalfa and vitamin E (wheat germ oil), or whole wheat germ. They found that the muscles showed progressive regeneration with ultimate restoration of the normal histologic structure.

Madsen, McCay and Maynard ¹¹ reported similar muscular changes in guinea pigs, rabbits, goats and sheep following administration of cod liver oil with normal and synthetic diets. On the basis of their investigations they concluded that the muscle lesions result from some toxic factor in the cod liver oil. Substitution of a vitamin A-D concentrate delayed the onset or lessened the severity of the degenerative changes but did not eliminate them. Reed ¹² commented on the histologic changes in skeletal muscle as well as in other tissues of the body in dogs following administration of a high concentration of vitamin D. He concluded that the degenerative changes observed in the tissues were due directly to the toxicity of vitamin D in large doses.

METHODS AND MATERIAL

Fifty guinea pigs weaned at the age of 21 days were used in the present study. After being weaned, they were kept for ten days on a carefully controlled adequate normal diet. In order that the effects of the various dietary factors might be studied, the animals were divided into six groups. The feeding experiments were run simultaneously, and all animals were kept in the same room and exposed to the same changes of temperature.

6. Hojer, A.: *Brit. J. Exper. Path.* **7**:356, 1926.

7. Meyer, A. W., and McCormick, L. M.: *Studies on Scurvy*, Stanford University Publications, University Series, Medical Sciences, Stanford University, Calif., Stanford University Press, 1928, vol. 2, no. 2.

8. Dalldorf, G.: *J. Exper. Med.* **50**:293, 1929.

9. Goettsch, M., and Pappenheimer, A. M.: *J. Exper. Med.* **54**:145, 1931.

10. Morgulis, S., and Spencer, H. C.: *J. Nutrition* **11**:573, 1936.

11. Madsen, L. L.; McCay, C. M., and Maynard, L. A.: *Memoir 178*, Cornell University, Agricultural Experiment Station, 1935.

12. Reed, C. Q.: *J. A. M. A.* **102**:1745, 1934.

Group 1. Animals 1-5 received 25 Gm. daily of the following normal diet:

Constituent	Parts
Alfalfa meal.....	15
Casein.....	5
Calcium carbonate.....	1
Sodium chloride.....	1
Butterfat (added daily).....	3
Orange juice—3 cc. daily	
Fresh raw carrots daily	

Group 2. Animals 6-14 received 25 Gm. daily of the synthetic diet described by Madsen, McCay and Maynard.¹¹

Constituent	Parts
Regenerated cellulose ¹³	20
Corn starch.....	40
Casein.....	15
Sucrose.....	10
Yeast.....	5
Lard.....	4
Salt mixture ¹⁴	4
Orange juice—3 cc. daily	
Carotene ¹⁵ —1 drop daily	
Brewers' yeast—0.2 Gm. added daily for each animal	

Group 3. Animals 15-23 received the normal diet as given group 1 and, in addition, 0.55 cc. of cod liver oil daily.

Group 4. Animals 24-32 received the synthetic diet as given group 2 and, in addition, 0.5 cc. of cod liver oil daily.

Group 5. Animals 33-41 received the synthetic diet as given group 2 and, in addition, were given daily an amount of viosterol^{15a} containing 500 U. S. P. units of vitamin D.

Group 6. Animals 42-50 received 25 Gm. daily of the diet described by Goettsch and Pappenheimer:⁹

Constituent	Parts
Rolled oats (Quaker).....	355
Wheat bran (Pillsbury).....	180
Skim milk powder (Merrell and Soule).....	75
Lard.....	80
Calcium carbonate.....	15
Sodium chloride.....	10
Cod liver oil—0.5 cc. daily	
Orange juice—3 cc. daily	

13. This product was purchased from the Sylvania Industrial Corporation, New York. It is a purified form of cellulose, washed free from glycerin. Before being used, the preparation was finely ground in a Wiley mill.

14. The salt mixture—used by Madsen, McCay and Maynard—was composed of:

Parts	Parts
K ₂ CO ₃ 219.0	HCl 28.5
Na ₂ CO ₃ 61.0	CaSO ₄ 0.2
CaCO ₃ 192.0	KI 0.03
MgCO ₃ 96.6	MgSO ₄ 9.12
Ferric citrate..... 20.0	NaF..... 0.372
FeSO ₄ 30.0	K ₂ Al ₂ (SO ₄) ₄ 0.037
H ₃ PO ₄ 41.8	Citric acid..... 546.1

15. The carotene used in this investigation was supplied by the S. M. A. Corporation, Cleveland.

15a. The viosterol used in this investigation was supplied by the Abbott Laboratories, North Chicago, Ill.

EXPLANATION OF FIGURE 1

Group 1. All animals in this group showed a consistent regular gain in weight. All survived the experimental period of eighty days. On gross and microscopic examination of the autopsy specimens, no abnormalities were noted.

Group 2. Only 2 of the 9 animals survived the eighty day period. The majority of the animals ultimately succumbed to infections of the respiratory tract. Autopsy revealed marked distention of the gastrointestinal tract, with petechial hemorrhages covering the serosal surface of the large intestines in many instances. The skeletal muscles showed no gross abnormalities.

Group 3. The animals in this group showed a less regular curve of growth than the animals in group 1, which did not receive the cod liver oil supplement. Two animals did not survive the experimental period. The skeletal muscles were extremely pale on gross examination. The original observations of Freeman and Farmer (*Am. J. Physiol.* **113**:1, 1935) concerning the presence of distended gallbladders in animals receiving large doses of cod liver oil were confirmed.

Group 4. The animals in this group did not respond favorably to the synthetic diet plus cod liver oil. None lived more than thirty days, and the majority died between ten and twenty-five days, after the beginning of the experiment. Three of the animals had profuse diarrhea for three to four days preceding death. One animal had bloody feces. Consolidation of the lungs resembling bronchopneumonia in man was observed in practically all of the animals in this group. As in group 3 the gallbladders were distended. Two of the animals showed extreme weakness of the hindlimbs several hours before death.

Group 5. One animal survived the eighty day experimental period. The majority showed a response similar to group 2 with the exception that the average life span was slightly longer. In the animals dying before the expiration of the experimental period pneumonic changes were the predominant pathologic features. As in group 4, several animals showed signs of irritation of the gastrointestinal tract. One showed weakness of the extremities so severe as to render its hind extremities powerless.

Group 6. The animals in this group manifested pathologic changes similar in character to those observed in groups 4 and 5. There was, however, little evidence of gastrointestinal irritation. Three animals showed extreme muscle weakness prior to death. Grossly the muscles appeared somewhat paler than normal. This was especially true in those animals dying of infections of the respiratory tract.

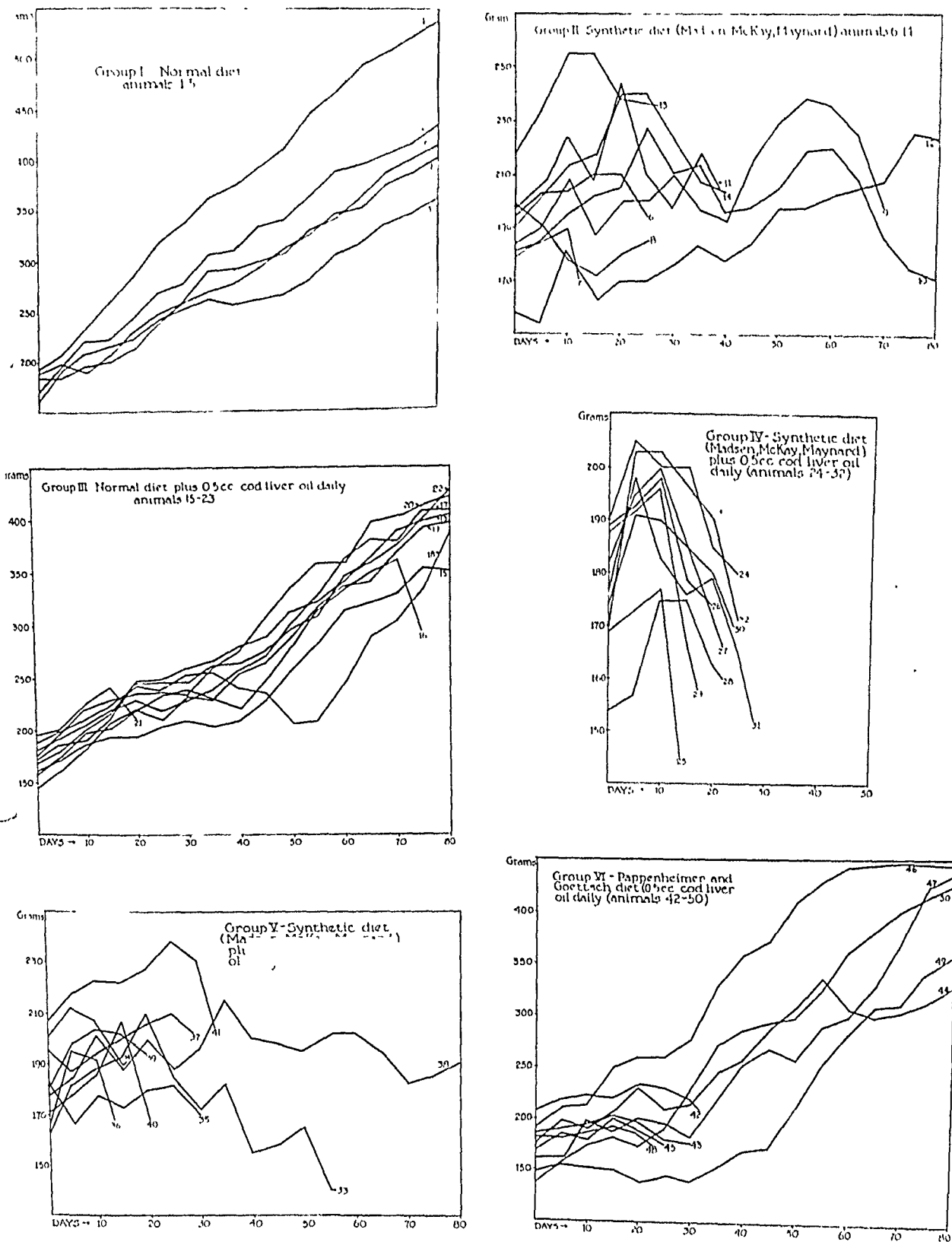


Figure 1

In addition to the foregoing animals, a special group of 9 guinea pigs 21 days old was used to ascertain the effect of starvation on muscle. These animals were given submaintenance amounts of an adequate diet. The amounts fed were progressively reduced so that the animals succumbed within fourteen to twenty-one days.

For the histologic studies of the skeletal muscle, the sections were stained by a modified Ranson^{15b} pyridine-silver, the hematoxylin-eosin, the Van Gieson and the Bodian^{15c} method.

RESULTS

The growth curves for the respective groups of experimental animals clearly demonstrate the response to the feedings of the various diets.

EXAMINATION OF SKELETAL MUSCLES

The gastrocnemius-soleus muscles were removed for examination when the animals were dying or immediately after death. The control animals all survived the experimental period of eighty days and were killed for examination.

Group 1—Normal Diet—Guinea Pigs 1-5.—All specimens were healthy and normal in appearance. Microscopically, there was no trace of any alteration in the muscle fibers, intramuscular nerves or blood vessels.

Group 2—Synthetic Diet (Madsen, McCay and Maynard)—Guinea Pigs 6-14.—Grossly, most of the muscles appeared normal. Microscopically, in some specimens there were irregularly scattered areas of damaged fibers. Many appeared swollen and granular. In these fibers the transverse striations were no longer visible. Instead a homogeneous dark-staining material caused the sarcolemma sheaths to bulge. Only small portions of individual fibers showed this necrosis, the remainder of the fiber being well striated and normal in appearance. In other portions, however, several adjacent fibers were necrotic throughout. A striking feature was the marked nuclear reaction. Many fibers were filled with several types of nuclei. Some were narrow and darkly staining, undoubtedly sarcolemmal; others were vesicular and resembled muscle nuclei; in addition, a great many irregular forms, phagocytic cells, could be distinguished. The process of myophagocytosis was present particularly in the severely necrotic areas, in some instances the degenerated fiber being completely replaced by a mass of these nuclei. There was also a proliferation of connective tissue cells in the areas of degeneration.

In some specimens there was a marked nuclear reaction without degeneration of the fibers. This early nuclear response is characteristic

15b. This stain was described by Chor.¹⁶

15c. Bodian, D.: Anat. Rec. 65:89, 1936.

of the reaction of striated muscle under pathologic conditions. These nuclei were largely sarcolemmal and muscle nuclei. The phagocytic reaction occurred later.

Group 3—Normal Diet and 0.5 Cc. Cod Liver Oil Daily—Guinea Pigs 15-23.—The muscles of this group were shrunk and pale with whitish streaks in some of the specimens. They were all extremely flabby. Microscopically, throughout the muscles were large and small areas of severely degenerated fibers in various stages of coagulation necrosis. Usually the damaged fibers were considerably swollen, but in other areas they appeared extremely narrow and atrophic. Increase in nuclei and myophagocytosis were evident.

Group 4—Madsen, McCay and Maynard Synthetic Diet and 0.5 Cc. Cod Liver Oil Daily—Guinea Pigs 24-32.—Many specimens were pale, shrunk and flabby. Microscopically, there were many swollen and degenerated fibers showing vacuoles and myophagocytosis. Multiplication of muscle and sarcolemmal nuclei was present. Little capillary reaction and only moderate infiltration with fibrous tissue were noted.

Group 5—Madsen, McCay and Maynard Synthetic Diet and Viosterol (500 Units)—Guinea Pigs 33-41.—Grossly, the muscles were shrunk, flabby and streaky in appearance. Microscopically, there were many areas of severely damaged fibers. Some were greatly swollen and contained a homogeneous granular substance without trace of cross striations. There was marked nuclear reaction and invasion by phagocytic cells. In other areas only portions of muscle fibers showed these degenerative changes, the remainder being healthy and normal in appearance. Many well preserved fibers were intermingled among the damaged ones. There was no prominent capillary reaction, although a few vessels showed endothelial hypertrophy. An increase in fibrous tissue was evident in some portions of the specimens. There was little reaction of the fat constituents.

Group 6—Goettsch and Pappenheimer Diet.—Grossly, the muscles were pale and flabby, with occasional streaks of white shining through. Microscopically, scattered irregularly throughout the specimen were dark-staining, swollen, degenerated fibers. Striations were no longer present in these fibers. The whole fiber was converted into a homogeneous granular material, resembling hyalin. In some of these areas there was an abundance of nuclei of different types. Some were clear and vesicular with little chromatin content; others resembled plasma cells and leukocytes. Muscle nuclei and sarcolemmal nuclei were definitely increased. This nuclear reaction was also present in fibers which were well preserved. This seems to indicate that this nuclear multiplication is a precursor of degeneration.

Group 7—Starvation Diet.—The muscles grossly were pale and flabby. Microscopically, many fibers showed fading of the cross



Fig. 2.—*A*, normal skeletal muscle (guinea pig 5); $\times 295$. The fibers are of uniform size, are well striated and show a normal distribution of muscle and sarcolemmal nuclei.

B, "nutritional myodegeneration" (guinea pig 9—Madsen-McCay-Maynard diet); $\times 135$. Many fibers are swollen and show waxy, hyaline degeneration. Some are completely necrotic and covered with nuclei of different types. Other fibers are extremely narrow and atrophic.

C, nutritional myodegeneration (guinea pig 44—Pappenheimer-Goettsch diet); $\times 250$. Many fibers are in a state of coagulation necrosis, with invasion of degenerated fibers by nuclei and phagocytic cells. It may be seen that portions of the fibers appear well striated. This picture resembles closely the degeneration of the rectus abdominis muscle in typhoid fever and pneumonia. (See Forbus,²¹ p. 323.)

D, nutritional myodegeneration (guinea pig 16—normal diet and cod liver oil). There are severe coagulation necrosis and degeneration of muscle fibers, in the midst of which may be seen well striated fibers.

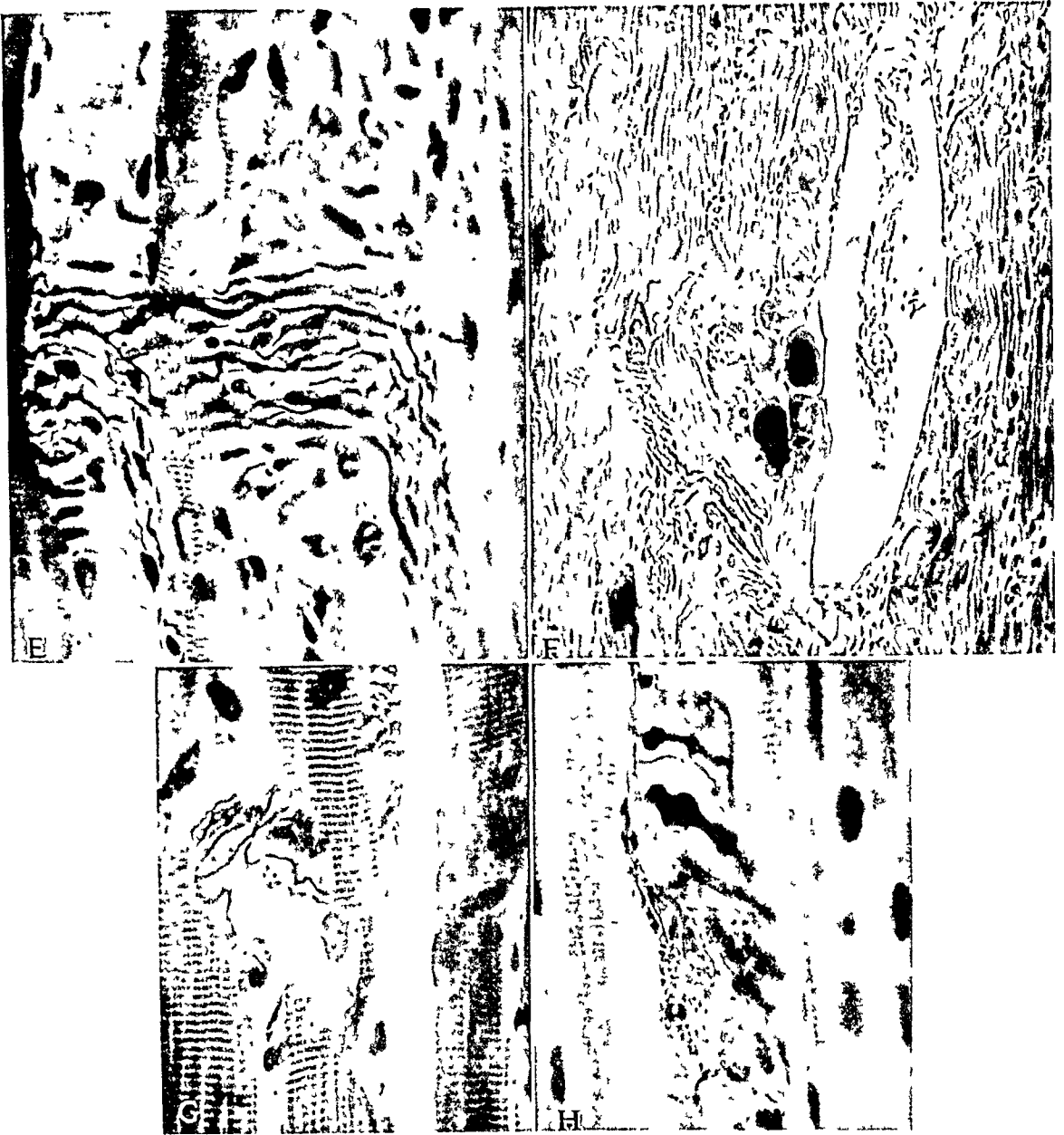


Fig 3.—*E*, nutritional myodegeneration (guinea pig 17—normal diet and cod liver oil). Well preserved intramuscular nerves and terminals in the midst of severely degenerated muscle fibers. This is in marked contrast to “nemogenic” atrophy in which nerve endings and intramuscular nerves show degeneration early before the muscle fibers break down.

F, nutritional myodegeneration (guinea pig 38—Madsen-McCay-Maynard diet and viosterol). Muscle spindle undergoing degenerative changes similar to those taking place in the extrafusal fibers. Note, however, the well preserved intramuscular nerves in the vicinity.

G, normal guinea pig muscle (guinea pig 5), showing normal motor nerve ending on well striated healthy fiber.

H, nutritional myodegeneration (guinea pig 17—normal diet and cod liver oil). Note irregular swellings on the terminal nerve fibers, which are still well preserved, supplying muscle fiber which is severely degenerated

striations. These fibers were narrow and pale but showed no definite necrosis or degeneration. There was no appreciable increase in nuclei. The blood vessels and the intramuscular nerves and their endings appeared normal. Fat cells were not found in any of the specimens.

THE MOTOR NERVE ENDINGS

The status of the motor nerve endings in various muscle disorders has received but little attention. This no doubt is due to difficulties in staining. In a previous publication¹⁶ a satisfactory method was described, in which a silver-pyridine stain is used. In the present study the Bodian staining method also has given excellent results. Changes in the motor nerve endings have been studied after nerve section and in experimental poliomyelitis. After nerve section degenerative changes are evident within twenty-four hours. These are ushered in by irregular swelling and staining of the fine terminal twigs of the neurofibrillar end brush. By the second day fragmentation of the neurofibrils appears, and the axon and myelin sheath outside the muscle fiber show irregularities in contour and in staining qualities. After the third day the ending is replaced by a granular strand which goes on to complete absorption, and the more proximal portions of the motor nerves show advanced fragmentation of axons and segmentation of myelin. Subsequently only remnants of nerve endings can be made out. These changes characterize "secondary" degeneration.

Kura and Kamesawa¹⁷ studied the motor nerve endings in mice inoculated with tetanus. They found swelling of the axis-cylinders, irregularity of the margins of these and an increase in tortuosity of the end ramifications. They emphasized the fact that the changes in the nerve endings which they described were primary changes and were noticeably different from the changes observed in secondary degeneration. Woolard¹⁸ studied the motor nerve endings in experimental beriberi. He found that the endings were swollen and bulbous and had lost their finer differentiation. He noted that the changes were most marked in the nerve endings and in the myelin and then in the axis-cylinder. These findings suggested that the disease exerts its effect at the peripheral endings.

The finding of well preserved intramuscular nerves and nerve endings in the midst of severely degenerated muscle is good evidence that the muscle changes are not secondary to damage to the lower motor neurons. In atrophy following nerve section and in poliomyelitis when the muscle degeneration is even moderate the nerve endings and intramuscular nerves are no longer to be seen.

16. Chor, H.: *Arch. Neurol. & Psychiat.* **29**:344, 1933.

17. Kura, N., and Kamesawa, S.: *Tr. Jap. Path. Soc.* **18**:330, 1928.

18. Woolard, H. H.: *J. Anat.* **61**:283, 1927.

Rogers, Pappenheimer and Goettsch¹⁹ studied the motor nerve endings in experimental "muscular dystrophy" in the guinea pig and found no alteration in the peripheral nerves or in the motor terminals. They studied also the larger peripheral nerve trunks and the central nervous system, which were found to be normal. They concluded, therefore, that the muscle changes were primary.

In the present study the intramuscular nerves were found to be well preserved in the midst of severely degenerated muscle. In some specimens, however, the terminal twigs of the telodendrion showed irregular swellings. Fragmentation or disintegration, however, was not found. The slight changes were apparently focal and were either the result of direct action of some toxic agent within the degenerating muscle fibers or a primary effect caused by some factor in the diet. This type of muscle lesion may be considered, therefore, to be extraneural in origin.

COMMENT

The nature of the histologic changes in skeletal muscle which occur in laboratory animals due to dietary factors differs greatly from those occurring as the result of lesions of the motor nervous system. The earliest reaction shows some similarity in that a multiplication of nuclei is the prominent feature in both. Later, however, the changes are quite distinct. The atrophic and degenerative changes in the muscle fibers cut off from their motor nerve consist in shrinkage of the fibers and a granular breakdown of the sarcolemmal elements, with replacement by fibrous tissue and fat. The intramuscular blood vessels also react by fibrosis of the vessel walls and endothelial hypertrophy. The intramuscular nerves and their endings completely disappear early in the process. The pathologic changes which result from deficiency in diet and from excess of cod liver oil are those of coagulative necrosis. Many fibers are voluminous and waxy in appearance. Some are narrow and shrunken. Fibrosis is not so prominent, and fat cells are rarely observed. No specific changes in the blood vessels can be detected.

It is interesting to note that in the groups of animals in which muscle lesions were observed the pathologic pictures were similar despite the different nutritional factors which produced them. The lesions obtained with the diet of Pappenheimer and Goettsch and with that of Madsen, McCay and Maynard were duplicated by the administration of cod liver oil to animals receiving a normal diet and by the addition of viosterol or cod liver oil to the synthetic diet of Madsen, McCay and Maynard. Rogers, Pappenheimer and Goettsch emphasized the fact that despite extensive degeneration of the muscle tissue the intramuscular nerves and their endings persist. Our observations are in agreement, although

19. Rogers, W. M.; Pappenheimer, A. M., and Goettsch, M.: *J. Exper. Med.* 54:167, 1931.

we did find slight changes at the very termination of the motor endings, which we think were due to a focal reaction.

The similarity between the pathologic changes observed in the present study and those described by Zenker²⁰ in typhoid fever and by Forbus²¹ in pneumonia is quite striking. The degenerative changes in pneumonia have been attributed to a toxic factor elaborated by the specific organism.

In our opinion the use of the term "dystrophy" to designate the muscle lesion resulting from nutritional deficiency may be confusing inasmuch as muscular dystrophy in man is a specific syndrome with characteristic clinical features. The word "dystrophy" is derived from the Greek *δυσ*—"badly" and *τρέφω*—"nourish." Etymologically, therefore, its usage in reference to conditions of malnutrition is correct. In human muscular dystrophy the muscle fibers show chronic degeneration with considerable replacement by fibrous tissue and fat. The status of the intramuscular nerves and their endings is still poorly understood. The disease occurs usually in childhood and is slowly progressive. There is a selective involvement of definite muscle groups rather than a generalized involvement of the musculature. The familial incidence and transmission through several generations suggest an underlying disturbance of the germ plasm. This is supported by the frequent association with other evidence of constitutional defect and inferiority, such as mental deficiency, epilepsy, optic atrophy, scleroderma and Friedreich's ataxia.

Because of certain similarities in the histologic and chemical aspects of these muscle lesions, there has been a tendency to apply the information obtained from experimental studies to the interpretation and treatment of human muscular dystrophies. We therefore suggest the use of the term "nutritional myodegeneration" rather than "dystrophy" for the muscle lesions produced by dietary alterations.

SUMMARY

Degenerative changes in skeletal muscle of guinea pigs have been produced by synthetic diets previously described by Pappenheimer and Goettsch and by Madsen, McCay and Maynard. These lesions were duplicated by the administration of cod liver oil to animals receiving a normal diet and by feeding animals the synthetic diet of Madsen, McCay and Maynard plus viosterol or cod liver oil.

The pathologic changes are those of coagulative necrosis. They closely resemble those described by Zenker in typhoid fever and by Forbus in pneumonia.

20. Zenker, F. A.: Ueber die Veränderungen der willkürlichen Muskeln im Typhus abdominalis, Erlangen, A. E. Junge, 1863.

21. Forbus, W. D.: Arch. Path. 2:318. 1926.

Despite marked degeneration of the muscle fibers, the intramuscular nerves and their terminals are well preserved. These observations are in agreement with those reported by Rogers, Pappenheimer and Goettsch. This type of muscle lesion may be considered, therefore, to be extra-neural in origin.

The histologic alterations characteristic of this experimental myopathy are different from those observed in muscular dystrophy in man. We suggest the use of the term "nutritional myodegeneration" rather than "dystrophy" for the muscle lesions produced by dietary alterations.

EFFECTS OF ORGANISMAL DIFFERENTIALS ON THE DISTRIBUTION OF LEUKOCYTES IN THE CIRCULATING BLOOD

HERMAN T. BLUMENTHAL, PH.D.
ST. LOUIS

NATURE OF THE PROBLEM

Two types of individuality may be distinguished according to Loeb:¹ (1) the mosaic type and (2) the type based on the presence of organismal differentials.

Each individual, with the possible exception of a unioval twin, differs from all others in a great variety of characteristics localized in the various tissues and organs of the body. These differences are structural as well as chemical and functional. Each individual is a mosaic of tissues and organs characterized by individual differences.

In addition, each individual organism, particularly if it belongs to the higher classes of animals, possesses a chemical characteristic which is common to all that individual's tissues and organs and differentiates it from all other individuals of the same species, each one of which also is distinguished by such a chemical denominator of the various tissue and organ constituents. These chemical characteristics distinguishing each individual of the same species have been designated by Loeb as individuality differentials. Correspondingly, species, orders and classes may be distinguished by their species, order and class differentials. All these various kinds of differentials, including the individuality differentials, comprise the organismal differentials. In an extensive series of transplantations carried out by Loeb and his associates² it could be

From the Laboratory of Research Pathology, Oscar Johnson Institute, Washington University School of Medicine.

These investigations were carried out with the aid of grants to Dr. Leo Loeb from the International Cancer Research Foundation and from the Josiah Macy Jr. Foundation.

These experiments formed the basis of a dissertation presented to the Board of Graduate Studies of Washington University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

1. Loeb, L.: *Science* **86**:1, 1937.

2. (a) Hesselberg, C., and Loeb, L.: *J. M. Research* **38**:33, 1918. (b) Loeb, L.: *ibid.* **6**:28, 1902; (c) **8**:44, 1902; (d) **39**:189, 1918; (e) **32**:353, 1918; (f) **41**:305, 1920; (g) *Biol. Bull.* **40**:143, 1921; (h) *Am. J. Path.* **2**:99, (i) 111, (m) 301 and (n) 315, 1926; (o) **3**:45, 1927; (p) *Arch. Path.* **10**:224, 1930; (q) *Physiol. Rev.* **10**:4, 1930; (r) *Biol. Bull.* **68**:440, 1935. (s) Loeb, L., and Addison, W. H. F.: *Arch. f. Entwcklungsmechn. d. Organ.* **27**:73, 1909; (t) **32**:44, 1911. (u) Loeb, L., and Harter, J. S.: *Am. J. Path.* **2**:521, 1926. Loeb.¹

shown that each one of the various types of transplantations—autogenous (transplantation of a piece of tissue into the same organism), syngenesious (transplantation into a nearly related animal), homogenous (transplantation into a nonrelated or distantly related animal of the same species) and heterogenous (transplantation into a different species)—is characterized by modes of reaction against the transplant on the part of the white blood cells, in particular the lymphocytes and polymorphonuclear leukocytes, and the connective tissue cells and blood vessels in accordance with the relationship of host and transplant. It is as if these various tissue elements belonging to the host recognized the genetic relationship between host and transplant and acted accordingly. It is especially the lymphocytes whose mode of reaction serves as the finest indication of individual differences as represented by the individuality differentials of host and transplant.

I shall now discuss briefly the nature of the interactions between host and transplant in accordance with their relationship, because a knowledge of these data is necessary for the understanding of the investigations on which I shall report subsequently. It may be assumed that a diffusion of certain substances from the graft into the host takes place, as a result of which certain kinds of cells of the host display a specific reaction against the transplant.

After autotransplantation lymphocytes are practically lacking around the graft, and connective tissue cells are attracted only to a moderate degree. The blood supply, on the other hand, is good because of the active ingrowth of capillaries into the transplant.

After homotransplantation there occurs around the transplant a reaction in which lymphocytes, connective tissue cells and blood vessels of the host are concerned. Lymphocytes are attracted by the tissue possessing a homodifferential; they collect around it, infiltrate it and injure it. The injury under certain conditions may progress to total destruction. Connective tissue cells also collect in increased numbers around the graft and invade it. Moreover, they tend to form dense hyaline tissue in contrast to their behavior in the autotransplant, where in contact with the transplanted cells they give rise to a cellular-fibrillar structure. The growth of capillaries into the homogenous transplant, and hence the blood supply of the latter, is much diminished in comparison with that which is characteristic of the autotransplant.

The reaction against the syngenesiotransplant stands midway between those observed after autotransplantation and homotransplantation. In comparison with the changes observed after homotransplantation the connective tissue reaction is, on the average, less marked; the lymphocytic reaction likewise is, on the average, diminished, but it varies in different cases in accordance with the genetic relationship between host and transplant. It may approach the autoreaction on the one hand, if the

genetic constitutions are very similar, and the homoreaction at the other extreme. All kinds of intermediate reactions have been observed in the experiments of Loeb.²⁰ In some cases, however, the diminution in the injurious action of fibroblasts, which is characteristic of syngenesious transplantation, may give the lymphocytes a chance to collect ultimately in larger numbers around the graft than in cases of homotransplantation, in which the marked new formation of fibrous tissue may injure the transplant at so early a stage that the chance of the lymphocytes to accumulate around it is diminished *pari passu* with the increase in the amount or in the toxicity of the homogenous substance which is able to diffuse into the host.

If the degree of relationship between host and donor is very far removed, as in the case of heterotransplantation, the reactions are more severe. Here the body fluids of the host are so different from those to which the transplanted tissue is adapted that they become markedly toxic for the latter; they become converted into heterotoxins and if the transplant is not very resistant to injuries, cause its death in a relatively short time. The connective tissue reaction of the host is very strong; besides, in an early period after transplantation polymorphonuclear leukocytes are attracted rather than lymphocytes, the latter arriving at a later period when the acutely acting heterotoxins have been largely absorbed.

The lymphocytes are the host cells which are most sensitive to differences between the individuality differentials of living tissues within the same organism. They are therefore very active in cases of syngenesiotransplantation and in cases in which the homodifferentials do not exceed a certain degree of difference. If the differences between the genetic constitutions of homogenous graft and host are very great, the connective tissue reaction tends to preponderate, and it may cause marked injury of the transplanted tissue at an early period. As stated, as a result of this injurious reaction, the lymphocytes do not find a chance to accumulate to the same extent as in the case of syngenesiotransplantation or of transplantation in which the differences between the homodifferentials of host and graft are less pronounced.

The body fluids of the host may also take part in reactions against homogenous tissues; but they are much less prominent in these reactions than in the reactions to heterotransplantation. It is especially the more sensitive tissues which are directly injured by the constituents of the strange homogenous body fluids.

It is clear, then, that the host cells can recognize a strange organismal differential and that, further, they can distinguish between different degrees of relationship between host and transplant. There is, however, a limit to this power of discrimination in that after a certain threshold of strangeness has been reached the reaction is maximal and

cannot be much increased by grafts of tissue from more distant donors. This applies to heterotransplantation.

At about the same time that experiments concerning the local reaction of the host against normal transplanted tissues were carried out by Loeb and his collaborators, somewhat comparable observations were made independently with transplanted tumors in rats and mice. Da Fano³ noticed in mice that after transplantation of a piece of carcinoma of the mammary gland lymphocytes and monocytes accumulated around the graft and at places distant from the tumor. He attributed to these cells the function of initiating immunity against the carcinomatous tissue. Furthermore, Tyzzer⁴ and Burgess⁵ observed around grafts of strange tumor tissue a collection of various leukocytes and increased activity of fibroblasts. They attributed these reactions to an inflammatory injury of the host tissue surrounding the tumor by anaphylo-toxin-like substances which developed in the host as the result of the tumor transplantation. Still another investigator, Baeslack,⁶ noticed that if a transplanted tumor grows continuously the polymorphonuclear leukocytes in the circulation of the host increase in number, whereas if the tumor retrogresses the lymphocytes increase in number. It was especially Murphy⁷ who, several years later, likewise stressed the behavior of the lymphocytes in the reactions against transplanted tumors. Also, according to this investigator, a general increase in the number of these cells is due to the development of immunity against the tumor graft. If the host resists the growth of the tumor and becomes immune, the lymphocytes in the lymph glands multiply more actively, and the lymphocytes in the general circulation increase in number; this does not occur if the host does not become resistant against the tumor and the latter grows successfully. He believed that if by treatment, such as the application of small doses of roentgen rays to the mouse, exposure of the animal to certain degrees of dry heat or the injection of certain oils into the mouse, an increase in the number of lymphocytes circulating in the vascular system is produced prior to transplantation of the tumor, immunity against the implanted tumor is increased. Under these conditions the animal is able to react even against autotransplanted pieces of tumor, i. e., against tissues which were derived from the host. By such means it is also possible to enhance resistance

3. Da Fano, C.: *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **5**:1, 1910.

4. Tyzzer, E. E.: *J. Cancer Research* **8**:109, 1916.

5. Burgess, A. W.: *J. M. Research* **16**:575, 1909.

6. Baeslack, F. W.: *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **20**:421, 1914.

7. Murphy, J. B.: *The Lymphocyte in Resistance to Tissue Grafting, Malignant Disease, and Tuberculous Infection: An Experimental Study*, Monograph 21, Rockefeller Institute for Medical Research, 1926.

against infection with tubercle bacilli in animals otherwise susceptible to infection with these organisms. The lymphocyte is then, according to Murphy, the main agent in the establishment of immunity against strange tumors, as well as against tumors derived from the animal's own cells, and also against certain pathogenic micro-organisms.

The majority of investigators assumed that the reactions against tumor grafts were due to immunity developing against the transplanted tissues and furthermore considered this immunity, or resistance, as specific for tumor. Yet the fact that a typical difference in the reactions of the host against the autotransplant and the homotransplant could be shown in the case of transplantation of tumor as well as in that of transplantation of normal tissues (Loeb^{2q}) and the fact that normal homogenous tissues produce immunity against homogenous tumors, while immunity against heterogenous tissues can be produced only through previous implantation of normal tissues derived from the same species (Ehrlich;⁸ Schoene⁹) suggested that the organismal differentials of normal tissues are also present in tumor tissues (Loeb^{2q}). It was therefore conceivable that also after transplantation of normal tissues changes in the constitution of the white blood cells might take place in the circulating blood and that these organismal differentials might diffuse not only into the tissues surrounding the graft but also into the general circulation and from there be carried to the blood-forming organs, where they would cause a stimulation of the lymphocytes or polymorphonuclear leukocytes in accordance with the kind of organismal differential active. I decided to determine, therefore, whether grafting of normal tissues was followed by characteristic changes in the relative and absolute number of white blood cells in the circulating blood corresponding to the local changes which were produced by the transplanted tissue. That diffusion of organismal differentials into the area of the host surrounding the graft takes place was indicated by an experiment of Crossen¹⁰ in which when lymph gland was autotransplanted in the vicinity of homotransplanted cartilage a movement of lymphocytes from the lymph gland to the cartilage could be seen to occur.

MATERIALS AND METHODS

The various tissues used in the experiments were grafted into subcutaneous pockets in guinea pigs, rats, pigeons or chickens as host animals. As a rule, the transplant was small, similar in size to a single lobe of the thyroid of a

8. Ehrlich, P.: *Ztschr. f. ärztl. Fortbild.* **3**:211, 1906; *Arb. a. d. k. Inst. f. exper. Therap. zu Frankfurt a. M.* **1**:77, 1906.

9. Schoene, G.: *Verhandl. d. deutsch. Gesellsch. f. Chir.* **36**:213, 1907.

10. Crossen, R. J.: *Arch. Path.* **6**:396, 1928.

guinea pig or rat. However, in some cases which I shall mention, multiple pieces were grafted simultaneously or in succession. Besides tissues, various nonliving substances were either implanted into subcutaneous pockets or injected in the form of solutions into guinea pigs and rats. Before and at various intervals after the transplantation, in the majority of cases every second day, differential counts of the white blood cells were made. In addition to the differential counts, total counts were made in a certain number of animals bearing autogenous, homogenous or heterogenous grafts.

In still other experiments excised organs were exposed to variations in temperature and to certain chemical substances for stated periods of time previous to transplantation in order that the effects of these factors on the organismal differentials of the transplanted tissues might be observed.

The differential counts were made by the dry smear method. A drop of blood from either the ear vein (guinea pig), the tail vein (rat) or a small superficial mammary vein (pigeon and chicken) was drawn out on a clean slide. This preparation was stained by the Wright blood stain method. Invariably 300 or more white cells were counted in each case.

Total counts were made with the Spencer Brite-Line hemocytometer according to the method described by Todd and Sanford.¹¹ Since eosinophils and basophils were present in very small numbers and were apparently unaffected by the experimental procedures, they were grouped with the neutrophils as polymorphonuclear leukocytes. It should be noted here that when the term "leukocytes" is used it is as a synonym for white blood cells and comprises lymphocytes as well as polymorphonuclear leukocytes.

CONTROLS

Differential counts made daily for from three to five days preceding transplantation served as control values. If the lymphocytes were the cells which showed an increase after grafting of a tissue, the highest percentage of lymphocytes found during the control period was taken as the base line for determining the effects exerted by the experimental procedures.

Certain extrinsic factors which are known to exert a considerable influence on the blood picture in both man and higher animals were taken into account both in arriving at control values and in interpreting the changes to be described.

In a series of experiments embracing 33 guinea pigs, 42 rats and 33 pigeons, differential counts were made on animals of various ages. The results are shown in table 1. It should be noted that as far as could be determined by gross examination these animals were free from infection.

From these results it appears that very young animals have greater lymphocyte counts than middle-aged ones. This comes out clearly in the case of guinea pigs in which it is seen that recently born animals have from 11 to 16 per cent more lymphocytes than young adults. In still older animals there is an increase. There seems to be a tendency toward increase of lymphocytes again in the peripheral circulation in pigeons and guinea pigs as these animals grow old. However, since only a relatively small number of old individuals of these three species could be obtained and since a corresponding curve was not observed in the case of rats, it may be that the variations in some of these cases were accidental.

11. Todd, J. C., and Sanford, A. H.: *Clinical Diagnosis by Laboratory Methods: A Working Manual of Clinical Pathology*, ed. 8, Philadelphia, W. B. Saunders Company, 1935.

It seemed that for the purpose of the experiments in view the most favorable age of the host animals in each of the three species was that of young adults. Rats weighing from 80 to 120 Gm. and guinea pigs weighing from 300 to 500 Gm. belong approximately to this class. The weight of the pigeons varied between 350 and 500 Gm. By carefully checking the weights of the animals which were used, it was possible to keep the age factor fairly constant as far as the host animals were concerned.

It is well known that leukocytosis follows ingestion of food, and there is, furthermore, general agreement that the maximum rise in the total leukocyte count occurs within two to three hours after a meal and may amount to as much as 100 per cent.

In order to avoid variations in counts due to food intake, specimens were invariably taken in the morning, when a period of approximately eighteen to twenty hours had elapsed since the last feeding.

TABLE 1.—*Influence of Age on Lymphocyte Count*

Animal	Number on Which Counts Were Made	Age	Average Percentage of Lymphocytes
Pigeon.....	10	2 days-4 weeks	69.5
	12	2 mo.-1 yr.	60.2
	10	1½-4 yr.	62.5
	1	5 yr.	69.0
Guinea pig.....	10	1 day-4 weeks	71.5
	10	6 weeks-6 mo.	55.8
	10	7 mo.-2 yr.	60.3
	3	3-5 yr.	72.0
Rat.....	12	1-4 weeks	63.3
	12	5 weeks-6 mo.	62.8
	15	7 mo.-2 yr.	58.3
	3	2½-3 yr.	57.0

Temperature effects must also be considered since Murphy and Sturm,¹² subjecting mice to dry heat at 55 to 65 C. for five minutes, found that both neutrophils and lymphocytes fell immediately after the exposure. Subsequently, however, the neutrophils recovered at a slow rate while the lymphocytes increased so rapidly that by the second week after heating the count often reached a point as high as 200 to 300 per cent above normal. I found an increase in lymphocytes in one set of control experiments carried out in the hot weather of early June 1936. These experiments were therefore discarded. All the other experiments were done at temperatures which were within the normal range; in these an increase in the lymphocytes due to a rise in temperature could be excluded.

Recently von Euler and Malmberg¹³ made comparative white blood cell counts of blood taken from the heart, the marginal ear vein and "pooled blood" of the guinea pig. They found the count of heart blood to be approximately one-third that of blood from the ear vein and the count of "pooled blood" two-thirds as high as that of blood from the ear vein. I attempted to repeat their experiments by making similar comparative counts of blood from the heart and from the ear vein every other day for a period of eight days. Thus each figure in table 2, which gives a summary of the results obtained, is an average of 4 counts.

12. Murphy, J. B., and Sturm, E.: *J. Exper. Med.* **29**:1, 1919.

13. von Euler, H., and Malmberg, M.: *Naturwissenschaften* **24**:713, 1936.

Here, too, there are consistent differences between the values for heart and ear vein blood, but these differences are not nearly as great as those found by von Euler and Malmberg, since the average count of blood from the heart is 90 per cent of the count of blood from the ear vein, as compared with approximately 33.33 per cent found by the latter investigators.

However, it was possible by modifying the mode of procedure to obtain differences between counts comparable to those obtained by von Euler and Malmberg. This modification consisted in allowing the heart blood to stagnate for a short time after its removal from the guinea pig.

Differential counts made simultaneously with the total counts in the same guinea pigs showed that the proportion of lymphocytes in blood taken from the ear vein was higher than that in blood taken from the heart. There were on the average 55 per cent lymphocytes in the heart blood as compared with 62.8 per cent in the blood taken from the ear vein.

TABLE 2.—*Comparative Counts of White Blood Cells in Blood from the Heart and from the Ear Vein*

Guinea Pig	Average Count of White Blood Cells per Cu. Mm. of Blood From Ear Vein	Average Count of White Blood Cells per Cu. Mm. of Blood From Heart
1.....	8,150	7,250
2.....	7,900	7,400
3.....	7,100	6,500
4.....	7,100	7,000
5.....	8,600	8,450
6.....	8,800	6,500
7.....	6,500	6,150
8.....	8,100	7,700
9.....	6,850	6,400
10.....	7,500	6,550
11.....	7,700	6,850
12.....	6,800	5,000
Average.....	7,591	6,813
	$6,813 / 7,591 = 0.90 \text{ (90\%)}$	

In order to avoid the difficulties shown by these experiments peripheral blood was used in all counts. As stated, in the case of guinea pigs blood from the marginal ear vein was used; in that of rats blood from the tail vein, and in that of pigeons and chickens blood from a superficial vein of the breast.

Further care was taken to eliminate all animals that were diseased and to discard all experiments in which the transplanted tissue showed any sign of infection.

NUMBER OF WHITE BLOOD CORPUSCLES IN THE CIRCULATION OF NORMAL ANIMALS

With the factors of nutrition, temperature, age and source of blood thus adequately controlled, both total and relative counts were made on a series of normal guinea pigs, while differential counts alone were made on a similar group of rats and pigeons, in order to determine the range of variation in the numbers of leukocytes circulating in the peripheral blood in normal animals that had not been operated on. The differential counts of the pigeon and rat were comparable. Since there is an inverse

ratio between changes in relative counts of lymphocytes and polymorphonuclear leukocytes (the proportion of lymphocytes rises while that of polymorphonuclear leukocytes decreases correspondingly) it is necessary to show only the variations in the number of lymphocytes. The average of the counts made on the 12 guinea pigs the first day represents the base line (O), and increases or decreases found in subsequent counts are measured by the height and position of the ordinates. The average increases and decreases found over a twenty day period were relatively slight. In the case of the lymphocytes the average deviation never exceeded 5 per cent in either direction; the average total counts, similarly, never showed a deviation of more than 500 in the positive and 600 in the negative direction. In only 2 guinea pigs did the increase in lymphocytes exceed 5 per cent; it rose to 8 per cent on the thirteenth day in one and to 10 per cent on the fifteenth day in the other. Similarly, deviations in the total counts greater than 500 in either direction were observed in only 3 animals. One showed an increase of 750 on the third day and the other 2 decreases of 800 and 750 white blood cells on the ninth day.

As far as the range of variations in the differential counts of pigeons and rats is concerned, it was very similar to that noted in guinea pigs.

EFFECTS OF TISSUE TRANSPLANTS ON THE ABSOLUTE AND
RELATIVE NUMBERS OF VARIOUS TYPES OF LEUKOCYTES
IN THE PERIPHERAL CIRCULATION OF THE HOST

Autotransplants.—In all, 42 experiments were made in which the effect of autotransplantation on the numbers of leukocytes in the peripheral blood was observed—24 with guinea pigs, 12 with rats and 6 with pigeons. Thyroid, testis, ocular lens and blood clot were used. As can be seen in table 3, in no case did the average maximum increase in percentage of lymphocytes exceed 6.5 per cent; if one excepts autotransplantation of blood clots, the average relative increase in lymphocytes was never greater than 4.8 per cent. Similarly, in the case of total counts as determined in 4 guinea pigs following autotransplantation of thyroid, the increase in no instance exceeded 500 white blood cells per cubic millimeter (table 4). However, in 3 single counts increases in percentage of lymphocytes did exceed 10 per cent. Two guinea pigs showed a 12 per cent and a rat a 10 per cent increase. Two of these exceptions occurred on the tenth day, the third on the sixteenth day, and they were probably due to some environmental factor. These high counts were, however, transient, since on the next occasion on which counts were made on these animals they showed an increase of less than 5 per cent.

It might be concluded that the variations found after autotransplantation were similar to those observed in normal animals. This is, however,

not the case, since in these experiments, with few exceptions, negative deviations were not observed. After autotransplantation there is generally an average maximum increase in percentage of lymphocytes of 5 per cent, and the average maximum increase in the total number of white blood cells is 500 leukocytes. In general there is a tendency for the counts to remain at the base line and occasionally to rise slightly above it during the course of a twenty day experiment. If the experiments with pigeons are omitted, only 12 of 378 counts made in the 36 cases of autotransplantation showed negative deviations, and these were

TABLE 3.—*Effect of Autotransplantation on Differential Counts of White Blood Cells in Peripheral Blood*

Animal Species	Experiments*	Tissue Transplanted	Average Maximum Percentual Increase of Lymphocytes	Average Day of Maximum Increase	Range of Variation in Percentual Increase of Lymphocytes	Range of Variation in Day of Maximum Increase
Guinea pig	12	Thyroid	4.1	6.1	1-12	1-11
	6	Blood clot	6.5	4.3	0-7	2-18
	6	Testis	4.8	5.5	4-6	2-6
Rat	3	Thyroid	3.3	11.0	2-10	5-16
	3	Blood clot	4.7	6.0	4-5	2-9
	6	Eye lens	4.3	5.7	2-7	3-9
Pigeon	6	Thyroid	0

* The total number of experiments was 42. In all the tables cases in which the transplants became infected were excluded.

TABLE 4.—*Effect of Autotransplantation on Total Counts of White Blood Cells in Peripheral Blood*

Animal Species	Experiments	Tissue Transplanted	Average Maximum Increase of White Blood Cells per Cu. Mm.	Average Day of Maximum Increase	Range of Variation in Increase of White Blood Cells per Cu. Mm.	Range of Variation in Day of Increase
Guinea pig	4	Thyroid	275	4.5	50-500	3-9

transient, the counts returning either to normal or to slightly above normal usually at the first, or at the latest at the second, subsequent count. The experiments with pigeons in which thyroid was autotransplanted differed from those with the other species inasmuch as the counts were very similar to those observed in controls, showing both slight positive and slight negative deviations.

Wounds; Inert Foreign Bodies: It was now necessary to determine whether this difference between the normal variations and the variations noted in bearers of autotransplants was due to the presence of the implanted tissue as such or whether it was due to nonspecific factors. Various inert substances, such as pieces of paraffin, silk thread

or agar, were therefore implanted into animals and differential counts made in the same way as in the experiments with autotransplantation. Table 4 shows the results. The positive deviations in animals with implanted inert substances were very similar in range to those in animals with autotransplants. There is, however, one minor exception to this conclusion. In 3 experiments in which paraffin was implanted changes similar to those observed in controls occurred, i. e., both small positive and small negative deviations. In additional experiments cuts were made in the skin of 4 guinea pigs, and these incisions were immediately closed by suture. Differential counts made on these animals were comparable in every way to those made after autotransplantation as well as after implantation of inert substances.

TABLE 5.—*Effect of Implantation of Inert Materials and of Operative Intervention on Blood Counts (Differential Counts)*

Animal Species	Experiments*	Substance Implanted	Average Maximum Percentual Increase of Lymphocytes	Average Day of Maximum Increase	Range of Variation in Percentual Increase of Lymphocytes	Range of Variation in Day of Maximum Increase
Guinea pig	4	Agar	6.6	10	5-7	8-12
	4	Thread	4.5	6.5	3-6	3-13
	16	Paraffin	3.4	6.8	0-6	1-18
	4	Cut and suture	4.5	5.5	2-8	1-19
Rat	4	Paraffin	2.0	4.5	3-5	1-4

* The total number of experiments was 32.

It is evident, then that simple operative procedures may act as a mild stimulant to the hemopoietic tissues, with the result that instead of observing a curve in the percentage of lymphocytes similar to that seen in controls, which is characterized by small positive and small negative deviations, one sees a number of small increases and almost no decreases. The time when the maximum of lymphocytic increase is observed is variable, ranging in both the case of autotransplantation and that of implantation of inert substances between one and eighteen days. The pigeon, however, appears to be refractory to mild stimulations such as those produced by autotransplantation and by the insertion of inert foreign bodies, since in all experiments of this kind the variations were the same as those previously observed in normal animals; also in 3 guinea pigs implantation of paraffin did not cause an increase in numbers of white blood cells.

One may therefore conclude that no changes in the white cell content of the peripheral blood could be attributed specifically to the presence of the autotransplanted tissue. The reactions observed were the result of nonspecific operative disturbances during the process of transplantation.

Homogenous Transplants.—In 253 experiments the effect of homotransplantation on the numbers of leukocytes was studied. As is shown in table 6, these transplantations included a wide variety of tissues. As usual, grafts were made into subcutaneous pockets with exception of 6 instances in which thyroids were transplanted to muscles in pigeons. Since the changes observed in these 6 cases did not differ from those seen after subcutaneous transplantation, they are included with the latter in table 6.

As can be seen from table 6, with few exceptions, to be discussed later, there is a marked increase in the proportion of lymphocytes in the differential count following homotransplantation. The increase is well above 10 per cent, and the day of maximum change shows much less variation than was found after autotransplantation or after implantation of inert substances.

After homotransplantation of thyroid (108 experiments), it was observed that by the fourth or fifth day there was a clearcut increase in lymphocytes. The maximum increase was usually reached on the sixth to seventh day; this was followed by regression to approximately the normal count. After homotransplantation of blood clot the reaction was more rapid. Definite increases in lymphocytes could be noted as early as the first or second day, while the maximum increase was generally observed on about the fourth day. In the case of the chicken the relative increase in lymphocytes was somewhat less marked and the time of the maximum increase somewhat later than in the other species, owing probably to the fact that the normal percentage of lymphocytes in the chicken is already very high (75 to 85 per cent). Under this condition a stimulus might be expected to be less effective than in the other three species.

Table 6 shows further that the increase in the average percentage of lymphocytes varies from 13.5 per cent in the pigeon to 16.9 per cent and 16.6 per cent in the rat and guinea pig, respectively, following homotransplantation of thyroid. Again, the pigeon generally has a higher normal proportion of lymphocytes than either of the other two species and the relative increase in lymphocytes under this experimental condition is therefore less great in the former.

It is interesting to note that 29 of the 35 experiments in which increases over 25 per cent were exhibited were carried out during the last two years of the investigation, while only 4 of the 35 experiments in which increases less than 25 per cent were shown were done during the same period. This makes it probable that with gradual improvement in technic the counts became slightly higher and that the average increase in lymphocytes in the homotransplantation experiments carried out in the course of three years was actually somewhat greater than the

figures given here indicate; however, these variations are not of a sufficient magnitude to alter the conclusions.

The total counts confirm essentially the results obtained with differential counts. After homotransplantation there is an increase in the total number of white blood cells per cubic millimeter of the peripheral blood, which reaches a maximum on the sixth to eighth day. This coincides approximately with the time at which the maximum increase

TABLE 6.—*Effect of Homotransplantation on the Distribution of Leukocytes in the Circulating Blood (Differential Counts)*

Animal Species	Tissue Transplanted	Experiments*	Average Maximum Percentual Increase of Lymphocytes	Average Day of Maximum Increase	Range of Variation in Maximum Percentual Increase	Range of Variation in Day of Maximum Increase
Guinea pig	Thyroid	68	16.6	7.1	11-31	4-12
	Liver	16	14.3	7.1	11-20	3-10
	Kidney	6	15.5	7.2	10-27	5-10
	Skeletal muscle	4	22.7	10.7	15-37	6-16
	Cardiac muscle	4	17.0	8.7	8-22	8-10
	Uterus	4	18.5	7.0	15-22	6-8
	Ovary	4	15.5	7.0	12-16	4-10
	Testicle	4	16.3	12.7	12-19	10-16
	Brain cortex	7	19.7	3.8	8-29	2-6
	Blood clot	6	20.5	4.2	8-30	3-6
	Plasma clot	10	18.2	5.2	15-25	2-6
	Cartilage	8	4.5	5.0	2-8	4-6
Rat	Thyroid	19	16.9	6.6	11-26	4-9
	Kidney	4	12.5	7.0	11-14	5-9
	Uterus	4	14.0	8.2	10-14	7-11
	Ovary	4	14.9	9.0	11-18	7-11
	Adrenal cortex	4	12.8	9.6	9-11	10-16
	Lymph node	4	14.8	10.0	10-20	9-11
	Lung	4	14.0	5.3	8-20	2-11
	Brain cortex	4	16.3	3.3	13-21	2-4
	Blood clot	4	16.3	3.3	11-21	2-4
	Decapsulated crushed lens	12	17.9	8.0	11-21	2-4
	Whole lens	8	6.3	8.0	5-11	2-14
	Crushed lens with capsule	4	16.5	7.5	12-20	5-10
	Tendon	4	7.1	10.3	5-8	7-14
	Cartilage	6	5.5	4.5	0-7	1-6
Pigeon	Thyroid	16	13.5	8.9	11-23	7-12
	Skin	6	9.3	12.6	7-12	8-12
Chicken	Thyroid	5	9.2	10.4	8-13	8-12

* The total number of experiments was 253. Included in this table are 14 experiments in which the counts were typical but in which the animals died before the experiments were concluded.

in lymphocytes as determined by means of differential counts occurs. Furthermore, in both the differential and the total counts the increase over the figures for the normal controls is approximately 16 per cent. If, as is shown in table 7, the absolute change in lymphocytes is calculated, the increase which takes place after homotransplantation is still more striking. There is an absolute average increase of 77.6 per cent in the case of the guinea pig and of 54.2 per cent in the case of the rat.

If all the tissues are classified according to the average time at which the maximum increase occurred, definite groups are found to exist. The earliest changes appear after homotransplantation of blood clot, plasma clot, brain cortex and lung, the maximum counts with these tissues being generally seen between the third and fifth day. However, increases in lymphocytes can often be noticed as early as the first or second day. The next group consists of homografts of thyroid, liver, kidney, uterus and crushed lens of the eye; here the maximum effect occurs usually between the sixth and eighth day. The ovary of the guinea pig also belongs to this group, while that of the rat belongs apparently to the next group, which consists of adrenal gland, lymph node, heart and skeletal muscle; with these organs the maximum increase is not reached until some time between the eighth and tenth day. In experiments with testicle and skin the maximum change

TABLE 7.—*Effect of Homotransplantation on the Distribution of Leukocytes in the Circulating Blood (Absolute Counts of Lymphocytes)*

Animal Species	Tissue	Experiments	Type of Count	Average Control Count	Average Maximum Count
Guinea pig	Thyroid	6	Total	7,775 leukocytes per cu. mm.	9,983 leukocytes per cu. mm.
			Differential	51.1% lymphocytes	70.7% lymphocytes
			Absolute	3,973 lymphocytes per cu. mm.	7,058 lymphocytes per cu. mm.
				7,058 — 3,973 = 3,085 $3,085 / 3,973 \times 100 = 77.5\%$ absolute increase in lymphocytes	
Rat	Thyroid	6	Total	9,083 leukocytes per cu. mm.	11,058 leukocytes per cu. mm.
			Differential	58.5% lymphocytes	74.1% lymphocytes
			Absolute	5,314 lymphocytes per cu. mm.	8,194 lymphocytes per cu. mm.
				8,194 — 5,315 = 2,880 $2,880 / 5,314 \times 100 = 54.2\%$ absolute increase in lymphocytes	

occurred even later, usually not before the twelfth day. There are, in addition, tissues which at no time following homotransplantation elicit the typical reaction. These include cartilage, tendon and intact lens of the eye. Transplantation of these yields results comparable to a certain extent, but perhaps not completely, to those obtained after autotransplantation or after merely operative interferences.

These variations in the time of the maximum reaction can be correlated with differences in the consistency of the various tissues, which apparently determine the readiness with which the effective substance is released from the grafts and reaches the hemopoietic organs in the host. In the first group, the tissues (blood clot, plasma clot and brain cortex) are very soft and loose, and the release is therefore greatly facilitated. On the other extreme are tissues such as cartilage, tendon, lens capsule and to a certain extent epidermis; these are hard tissues, which tend to prevent diffusion of the active substance. In addition, one has to consider the possibility that in these firmer tissues the

metabolism is low and that correspondingly the individuality differentials may be produced in only small amounts. In the case of cartilage the lack of a homoreaction has been confirmed by total counts. Intermediate between these two types of tissues are the glandular and muscular tissues, in which the maximum release of active substances takes place later than in the first group but with which the maximum counts are very high. The maximum reaction is reached somewhat more rapidly with glandular tissues than with muscle. The difference between ovaries of the rat and guinea pig may perhaps be due to the fact that in the ovary of the rat the corpora lutea are much more numerous than in the ovary of the guinea pig.

If one considers only the relative counts of lymphocytes, the degree of maximum increase of these cells shows no apparent correlation with the time at which this increase is reached. But an analysis of the absolute counts (table 6) shows that in those instances in which the maximum increase appeared on the fifth or sixth day after homotransplantation the increase was greater than in those in which the maximum increase was reached later, namely, on the eighth or ninth day; in the former the ascent of the curve representing this increase in the absolute number of lymphocytes was steeper than in the latter cases.

The fact that it was possible to observe typical generalized homoreactions following transplantation of homogenous blood clots or plasma clots is of particular interest since Loeb¹⁴ could find only slight differences histologically between the reactions against autogenous and homogenous blood clots. The differences between the local and general reactions against blood clots are presumably due to the great rapidity with which the individuality differentials are extracted from this material, as is indicated by the very early appearance of the increase in lymphocytes in the general circulation; under this condition the local reaction has not had a chance to become manifest in full strength. The response on the part of the leukocytes in the circulation appears thus to be a very sensitive test for the action of a homotoxin. Furthermore, since the typical lymphocytic reaction in the peripheral blood occurred also after homotransplantation of plasma clot, constituents of the blood other than the red cells may also contain the organismal differentials.

The occurrence of a typical peripheral blood response to homotransplantation of crushed ocular lens is of particular interest. Uhlenhuth¹⁵ showed on the basis of immunologic tests that the protein

14. Loeb.^{2e} Loeb.²ⁿ

15. Uhlenhuth, P., in *Festschrift zum sechzigsten Geburtstage von Robert Koch*, Jena, Gustav Fischer, 1903, p. 49.

of the lens of the eye is organ specific but lacks species specificity almost entirely. The findings of Uhlenhuth have been confirmed and further extended by Hektoen and Schulhof.¹⁶ Fleisher¹⁷ used transplantation as a test for the presence of the individuality differential in this tissue. He was unable to observe any marked difference between the local lymphocytic reactions around autotransplanted and homotransplanted lens and concluded, therefore, that this tissue did not seem to possess an individuality differential. In accordance with a suggestion made by Loeb, Fleisher considered the possibility that the capsule of the lens presented a mechanical barrier to the interchange of substances between host and transplant and to the entrance of lymphocytes and fibroblasts of the host into the homogenous transplant, but he dismissed this possibility because the reaction around the lens was apparently lacking even in cases in which the capsule had been accidentally broken. My experiments with whole lens were likewise negative. On the other hand, crushed lens, whether previously decapsulated or not, always gave a typical homoreaction. Similarly in a case in which the whole lens was accidentally crushed during the process of transplantation a positive result was obtained. Thus, from my experiments it would seem that Loeb's explanation is the correct one. It is possible that in Fleisher's experiments the injury to the capsule was not sufficient to offer enough surface contact between lens fibers and host tissue or that the absorption of the active substance was too rapid to make possible a local reaction.

These experiments show, therefore, that the individuality differential is present in a wide variety of tissues of the body, a fact already well established by Loeb,¹⁸ and that when these tissues are homotransplanted there takes place a typical general reaction, characterized by both an absolute and a relative increase in the number of lymphocytes in the peripheral blood, which corresponds to the local reaction about the transplanted tissue. This general reaction in the presence of homogenous tissue occurs only when a threshold quantity of homotoxin is released, a lesser amount eliciting merely a wound reaction. On the other hand, it seems that a greater than threshold quantity does not increase the general reaction to a noticeable extent. This was demonstrated by the fact that 6 guinea pigs in which multiple homotransplantations of thyroid gland were carried out simultaneously—2 animals receiving two, 2 others four and the last 2 six thyroid grafts—did not show a greater percental increase of lymphocytes than was observed following a single homogenous transplantation of thyroid, nor did the

16. Hektoen, L., and Schulhof, K.: *J. Infect. Dis.* **34**:433, 1924.

17. Fleisher, M. S.: *J. M. Research* **42**: (a) 173 and (b) 491, 1921.

18. Loeb.^{2d} Loeb.^{2d}

reaction appear earlier under these circumstances. It seems that the reaction requires a certain threshold amount of tissue; if this is exceeded the reaction is neither intensified nor accelerated.

Syngenesiotransplants.—Fourteen experiments were carried out in which thyroids were syngenesiotransplanted in guinea pigs, the tissues being transferred to brother or sister. The average maximum percental increase of lymphocytes was 11.7, distinctly less than that observed after homotransplantation. The variations in the maximum increase of lymphocytes ranged between 8 and 18 per cent. In 4 of these 14 experiments the increase was less than 10 per cent, while in only 2 it was above 15 per cent. Not only was the average maximum increase in the number of lymphocytes less after syngenesiotransplantation than after homotransplantation, but also the average time at which this maximum increase was reached was greater, namely, twelve and one-tenth days as compared with seven and one-tenth days in the corresponding homotransplantations. The time at which the maximum

TABLE 8.—*Effect of Syngenesiotransplantation on the Distribution of Leukocytes in the Circulating Blood (Differential Counts)*

Animal Species	Tissue Transplanted	Experiments	Average Maximum Percental Increase of Lymphocytes	Average Day of Maximum Increase	Range of Variation in Maximum Percental Increase	Range of Variation in Day of Maximum Increase
Guinea pig	Thyroid	14	11.7	12.1	8-18	6-19

increase occurred varied between six and nineteen days. Furthermore, in the 4 cases in which a maximum increase in lymphocytes of less than 10 per cent was shown the increase was reached between the sixth and nineteenth day after transplantation, whereas in the remaining 10 cases variations in time ranging from the sixth to the thirteenth day were exhibited. Thus some of the syngenesiotransplants elicited reactions similar to those observed after homotransplantation, while the reactions to others closely resembled those following autotransplantation. The largest group, consisting of 8 cases in which the maximum increase was somewhat less than that observed after homotransplantation and in which it usually appeared at a later time, behaved in an intermediate manner.

One can thus observe following syngenesiotransplantation reactions of intensities ranging from that observed after autotransplantation, at one extreme, to that seen following homotransplantation, at the other, with the greatest number of experiments giving intermediate responses. There are quantitative differences between the reactions observed after homotransplantation and those following syngenesiotransplantation. In

the latter a longer time is generally necessary for the release of a quantity of the strange organismal differentials sufficient to induce a general reaction.

Heterotransplants.—As shown in table 9, altogether 107 transplantations of a variety of heterogenous tissues were carried out. The most striking difference between the results in these experiments and those observed following homotransplantation consists in the kind of white blood cells which react. Whereas after homotransplantation there is both a relative and an absolute increase in the lymphocytes of the peripheral blood, grafting of heterogenous tissues is followed by a primary increase in the number of polymorphonuclear leukocytes and, again, this increase is both a relative and an absolute one.

Furthermore, the reaction to heterogenous grafts tends to appear a few days earlier than does the reaction to homogenous transplants (table 9). Following heterotransplantation of thyroid the increase in polymorphonuclear leukocytes reaches a maximum on about the fifth day; this is approximately two days earlier than that observed after homotransplantation. The beginning of the increase can be noticed already on the second or third day after heterotransplantation of thyroid; this corresponds to the time when the increase of lymphocytes becomes noticeable in the cases in which the easily absorbed blood clot is homotransplanted. This increase in polymorphonuclear leukocytes is followed by a regression which continues until approximately the normal count is attained, usually about the tenth day. Subsequently there takes place a secondary reaction characterized by an increase, both relative and absolute, in the number of lymphocytes, which usually reaches a maximum between the fourteenth and sixteenth days and again is followed by a regression to normal.

As was observed in the case of homotransplantation, there are differences in the time of reaction which depend on the kinds of tissue used for heterotransplantation. Here, too, blood clot and brain give the earliest reactions, the glandular tissues intermediate ones, and heterotransplanted cartilage and tendon, which after homotransplantation gave no reaction at all, produce a definite change characterized by a relatively late appearance of the maximum increase, usually about the seventh day. Crushed lens also belongs to the category of tissues giving a tardy reaction.

Likewise, after heterotransplantation of plasma clot a typical reaction is obtained; as after homotransplantation, it is later in its appearance than that following heterotransplantation of whole blood (table 9). It thus seems that the substances acting as, or given origin to, homotoxins and heterotoxins are present in the red blood corpuscles as well as in the fibrin, but the latter contains an amount sufficient only to cause a retarded reaction.

Furthermore, tissues such as adrenal, and in the rat also ovary, which called forth fairly late maximal responses after homotransplantation react similarly following heterotransplantation, while testicle, which caused a relatively late change when homografted, induces a heteroreaction within the same period of time as do glandular tissues such as thyroid, liver and kidney. It should also be noted that crushed lens is capable of producing a typical heteroresponse and contains, therefore, heterodifferentials as well as homodifferentials.

TABLE 9.—*Effect of Heterotransplantation on the Distribution of Leukocytes in the Circulating Blood (Differential Counts)*

Animal Species	Tissue Transplanted	Experiments*	Average Maximum Percent Increase of Polymorphonuclears	Average Day of Maximum Increase	Range of Variation in Percent Maximum Increase	Range of Variation in Day of Maximum Increase
Pigeon to guinea pig	Thyroid	9	16.6	5.6	13-23	5-6
	Cartilage	4	16.0	6.0	12-23	5-6
	Blood clot	4	26.7	3.5	23-34	2-5
Guinea pig to pigeon	Thyroid and cartilage	4	18.8	5.0	15-23	4-6
Rat to guinea pig	Thyroid	10	19.2	4.9	12-29	3-10
	Cartilage	6	13.8	6.8	11-16	6-8
	Blood clot	6	19.5	3.5	12-28	1-5
Guinea pig to rat	Thyroid	4	16.8	5.0	8-23	4-6
	Uterus	4	13.0	5.0	10-16	4-6
	Ovary	4	18.5	9.0	14-25	8-10
	Adrenal cortex	4	17.5	7.0	14-20	6-8
	Testicle	4	12.0	5.5	9-14	2-8
	Brain cortex	4	18.0	4.5	11-24	2-8
	Blood clot	4	15.5	3.5	10-23	2-5
	Decapsulated crushed lens	8	20.6	7.3	12-32	4-10
	Tendon	4	13.3	4.5	12-15	2-6
	Lymph node	4	14.5	4.5	9-20	4-6
Mouse to rat	Cartilage	4	12.3	5.0	9-17	4-6
	Liver	4	14.3	5.0	12-17	4-8
	Kidney	4	20.0	5.0	13-29	4-8
Rabbit to guinea pig	Plasma clot	4	15.3	5.0	9-19	4-8
Rabbit to rat	Plasma clot	4	22.0	7.5	18-25	6-8

* The total number of experiments was 107.

The results of total counts as determined in a series of 8 experiments parallel those obtained by means of differential counts. However, when the absolute increase in the number of polymorphonuclear leukocytes is determined from these data, it is seen that this increase is much greater than that indicated by the differential counts, since the magnitude of change exceeds 100 per cent. Furthermore, the absolute increase of polymorphonuclear leukocytes which takes place in response to heterotransplantation is much greater than the absolute increase of lymphocytes in the case of homotransplantation. The reaction following heterotransplantation is therefore more acute and severe than that following homotransplantation. In the former it is the

polymorphonuclear leukocytes which react rather than the lymphocytes; the rapidity with which the reaction takes place is greater, and the magnitude of the increase exceeds that occurring after homotransplantation.

The secondary increase in lymphocytes previously mentioned, which occurs between the tenth and fourteenth days, parallels closely, as far as magnitude is concerned, the primary increase following homotransplantation. In the 4 experiments in which the absolute increase in polymorphonuclear leukocytes after transplantation of rat tissues into guinea pigs had been determined, the secondary average maximum increase in lymphocytes amounted to 14.5 per cent; it occurred, on the average, fourteen and six-tenth days after the operation.

Finally experiments were carried out in which pieces of mouse carcinoma which had been serially propagated by means of homografts

TABLE 10.—*Effect of Heterotransplantation on the Absolute Counts of Polymorphonuclear Leukocytes in the Circulating Blood*

Animal Species	Tissue Trans-planted	Experi-ments	Type of Count	Average Control Count	Average Maximum Count
Rat to guinea pig	Thyroid	4	Total	6,988 leukocytes per cu. mm.	9,050 leukocytes per cu. mm.
			Differential	32.0% polymorpho-nuclears	51.8% polymorpho-nuclears
			Absolute	2,236 polymorphonuclears per cu. mm.	4,688 polymorphonuclears per cu. mm.
			4,688 - 2,236 = 2,452 $2,452 / 2,236 \times 100 = 109.6\%$ absolute increase in polymorphonuclear leukocytes		

were transplanted into 4 rats and 4 guinea pigs. The differential counts showed a typical heteroreaction in each case consisting in an early increase in lymphocytes. Five of the animals exhibited the maximum secondary lymphocytic reaction between the seventh and tenth days, while the remaining ones showed typical secondary reactions between the tenth and fourteenth days.

EFFECT OF SUCCESSIVE TRANSPLANTATIONS ON THE DISTRIBUTION OF LEUKOCYTES IN THE PERIPHERAL BLOOD

Successive transplantations of pieces of tumors into the same host and other experiments of a related nature have shown that by this procedure the resistance of the host against the grafted tumor can be increased. According to Lumsden¹⁹ and Woglom,²⁰ immune substances can be demonstrated in the blood serum of animals actively immunized against a tumor or in which an implanted tumor retrogresses apparently

19. Lumsden, T.: *Am. J. Cancer* **15**:563, 1931.

20. Woglom, W. H.: *Cancer Rev.* **4**:129, 1929; *Am. J. M. Sc.* **181**:157, 1931.

spontaneously. Furthermore, Schoene²¹ carried out a series of experiments in which he attempted to demonstrate that immunity developed against a graft of normal tissue (skin) as a result of a preceding injection of a suspension of liver, spleen or kidney. In his experiments the tissue used for immunization belonged to the same species as the subsequently grafted skin. In homotransplantation in rabbits he believed that he had succeeded in producing immunity; in mice, on the other hand, his attempts proved to be unsuccessful. Because of the many variable factors which enter into the success of a skin graft, and since in the large majority of cases a homogenous skin graft remains united with the host only temporarily, these results are not quite convincing as far as homotransplantations are concerned. In the case of successive heterotransplantations of mouse skin on rats, on the other hand, Schoene's results seem more convincing.

Loeb²² approached this problem by means of successive homotransplantations of the same type, as well as of other types, of tissue and could find no definite acceleration of the reaction against a second homotransplant as compared with that against a first graft. Fleisher^{22a} also found that previous immunization of an animal by injection of suspensions of homogenous tissue did not alter the nature or the course of the reaction which took place around a homotransplant. However, in the case of heterotransplantation he^{22b} did find an increased reaction on the part of polymorphonuclear leukocytes, which surrounded and invaded the graft in the first few days in an animal immunized against tissues of that particular species; in addition, he noticed a delay in the ingrowth of fibroblasts and interference with the slight growth processes which may take place in the heterogenous tissue.

In order to determine whether acceleration of the reaction following a second transplantation could be detected by means of blood counts, a series of 82 experiments was carried out in which two successive homotransplantations were made, and in 23 additional experiments the effects of two successive heterotransplantations on the number of leukocytes were determined. In the case of the homotransplantations a first subseries of experiments consisted of 58 in which an interval of ten days intervened between the two transplantations while in a second subseries, consisting of 24 experiments, the length of the intervening period was twenty-one days. Twelve experiments in each group served as controls; in these, inert paraffin took the place of the first tissue graft. Rats as well as guinea pigs were used as hosts.

Successive Homotransplantations.—Homotransplantations of thyroid after a previous implantation of paraffin called forth a typical homo-

21. Schoene, G.: München. med. Wchnschr. **59**:457, 1912.

22. Fleisher, M. S.: (a) J. M. Research **38**:191, 1918; (b) **37**:483, 1918.

reaction as far as the magnitude of increase and the average time of maximum reaction were concerned. On the other hand, if the first transplant consisted of living homogenous tissue, the reaction following the subsequent transplantation of the same or of another kind of tissue of the same species was definitely accelerated; it occurred from two to four days earlier than the reaction after the first grafting. As is shown in table 11, there is no difference between the results obtained in the first subseries, in which the interval between the two transplantations was ten days, and the results obtained in the second one, in which the interval was twenty-one days.

Despite the acceleration of the reaction, the actual increase in the number of lymphocytes following the second transplantation was less than that following the first transplantation in all cases except 5 (noted in part 1 of table 11) in which the increase following the second grafting was greater than that observed after the first.

Three experiments in which four successive homotransplantations of blood clot were made into guinea pigs at four day intervals failed to reveal any diminution in the number of lymphocytes after the second, third or fourth transplantation nor was there any evidence of a more rapid appearance of the lymphocytic increase. This is probably at least partly due to the fact that the reaction to a first implantation of blood clot is already a very rapid one; the maximum increase occurs usually between the second and the fourth day.

In addition to the experiments shown in table 11 there was a series of 8 experiments—4 in guinea pigs and 4 in rats—in which not only differential counts but also total counts were made. The changes established by means of the latter closely paralleled the results of the differential counts. In the guinea pigs there was a maximum average increase of 1,585 leukocytes per cubic millimeter of peripheral blood following the primary transplantation, and in the rats, a corresponding average increase of 2,010 leukocytes. The increases following the second transplantations were on the average 1,150 leukocytes in the guinea pigs and 1,220 in the rats. The average period of time preceding the primary maximum increase was seven and one-tenth days in the guinea pigs and six and eight-tenth days in the rats, whereas the average period preceding the secondary maximum increase was four and five-tenths days in the former and four and three-tenths days in the latter.

Successive Heterotransplantations.—The reactions of the leukocytes to successive transplantations of heterogenous tissues are shown in table 12; they are quite comparable to those following successive transplantations of homogenous tissues except that the changes primarily involve the polymorphonuclear leukocytes instead of the lymphocytes.

With both kinds of successive transplantations the maximum average increase in leukocytes was less after the second than after the first transplantation, but this maximum reaction was reached at an earlier time. The reactions were not noticeably affected by a preceding implan-

TABLE 11.—*Changes in Counts of Lymphocytes Following Successive Homotransplantations*

Animal Species	Experiments*	Tissue Transplanted	Average Maximum Percentual Increase of Lymphocytes	Average Day of Maximum Increase	Range of Variation in Percentual Increase	Range of Variation in Day of Maximum Increase
1. Interval of Ten Days Between First and Second Transplantation						
Guinea pig	8 control experiments	1—paraffin	4.8	1.5	0-5	4-10
		2—thyroid	16.4	6.0	8-27	4-8
	9	1—thyroid	21.0	7.3	13-28	6-8
		2—thyroid	18.7	3.6	11-29	2-4
	8	1—thyroid	17.0	7.9	10-29	8-10
		2—liver	14.3	4.0	9-25	2-4
	6	1—liver	14.3	7.3	10-20	4-10
		2—liver	13.7	4.0	11-25	2-4
	6	1—liver	12.7	6.3	10-16	5-7
		2—thyroid	11.3	4.0	8-19	2-4
	4	1—kidney	11.5	6.5	10-15	5-7
		2—thyroid	10.5	4.5	7-15	3-7
	4	1—kidney	14.5	8.5	11-18	7-10
		2—kidney	10.0	4.0	8-12	2-4
4	1—liver	14.0	7.5	12-17	3-10	
	2—kidney	11.5	5.0	8-17	2-10	
2	1—thyroid	17.5	6.0	14-21	Both 6	
	2—kidney	14.5	4.0	14-15	Both 4	
Rat	4 control experiments	1—paraffin	4.5	2.5	3-5	2-4
		2—thyroid	17.5	6.0	11-19	4-8
	1	1—thyroid	18.0	8.0		
		2—thyroid	11.0	4.0		
	2	1—thyroid	13.5	6.0	13-14	Both 6
		2—liver	11.0	4.0	9-13	Both 4
2. Interval of Twenty-One Days Between First and Second Transplantation						
Guinea pig	8 control experiments	1—paraffin	4.0	7.5	3-6	1-16
		2—thyroid	11.0	6.9	2-21†	5-8
	8	1—thyroid	14.3	7.3	11-18	5-8
		2—thyroid	11.5‡	3.0	8-15	2-4
	4 control experiments	1—paraffin	4.3	8.3	3-6	1-12
		2—thyroid	15.5	6.5	11-22	5-8
	4	1—thyroid	17.0	8.0	11-17	6-9
		2—thyroid	12.3	4.0	9-15	2-5

* The total number of experiments in group 1 was 58; that in group 2 was 24.

† One animal failed to react to the thyroid transplant.

‡ One animal showed a greater increase after the second transplantation than after the first, but this increase appeared as early as on the second day after the second transplantation as compared with the maximum increase on the eighth day after the first transplantation.

tation of an inert foreign substance, such as paraffin. In addition to the differential counts, a number of total counts were also made in this series; these are shown in table 13. The total counts closely paralleled the differential counts.

Not only is the increase in polymorphonuclear leukocytes accelerated in the case of a second heterotransplantation, but the later phase of the

reaction which takes place after heterotransplantation, consisting of an increase in lymphocytes, is also accelerated. Instead of occurring between the tenth and fourteenth days it usually takes place between

TABLE 12.—*Changes in Differential Counts of Polymorphonuclear Leukocytes Following Successive Transplantations of Heterogenous Tissues*

Animal Species	Experiments*	Tissue Transplanted	Average Maximum Percent Increase of Polymorphonuclears	Average Day of Maximum Increase	Range of Variation in Percent Increase	Range of Variation in Day of Maximum Increase
Rat to guinea pig	4 control experiments	1—paraffin	20.5	5.3	12-30	4-6
		2—thyroid	25.2	5.8	12-29	4-10
	7	2—thyroid	13.4	3.0	9-34	2-4
		1—thyroid	21.0	6.0		
	1	2—kidney	12.0	4.0		
		1—cartilage	14.8	5.3	11-23	4-8
	6	2—thyroid	10.0†	3.3	7-16	2-4
		1—thyroid	18.0	6.0		
	1	2—liver	13.0	4.0		
		1—thyroid	12.5	6.0	12-13	Both 6
Guinea pig to rat	2	2—liver	8.5	4.0	5-12	Both 4
		1—thyroid	19.0	6.0		
	1	2—thyroid	12.0	4.0		
		1—thyroid	10.0	6.0		
	1	2—kidney	5.0	2.0		

* The total number of experiments was 23.

† In three cases an increase in the relative number of polymorphonuclear leukocytes of less than 10 per cent followed the second transplanation.

TABLE 13.—*Changes in Total Counts of Leukocytes Following Successive Transplantations of Heterogenous Tissues*

Animal Species	Experiments*	Tissue Transplanted	Average Maximum Increase of Leukocytes per Cu. Mm.	Average Day of Maximum Increase	Range of Variation in Maximum Increase	Range of Variation in Day of Maximum Increase
Rat to guinea pig	2	1—thyroid	1,950	6.0	1,550-2,350	Both 6
		2—thyroid	1,100	4.5	900-1,300	4-5
	1	1—thyroid	2,200	5.0		
		2—kidney	1,450	4.0		
	1	1—thyroid	1,750	6.0		
Guinea pig to rat	2	2—liver	900	4.0		
		1—thyroid	2,350	5.5	2,000-2,700	5-6
	1	2—liver	1,200	3.5	900-1,500	3-4
		1—thyroid	1,900	5.0		
	1	2—thyroid	950	4.0		
	1	1—thyroid	1,750	6.0		
		2—kidney	1,200	2.0		

* The total number of experiments was 8.

the seventh and tenth days. In all my experiments the second heterotransplantation was carried out ten days after the first grafting, at a time, therefore, when the increase in lymphocytes caused by the pri-

mary graft should have been just beginning. But this increase in lymphocytes caused by the primary graft was never actually observed, being apparently obliterated by the increase in polymorphonuclear leukocytes caused by the second transplant. The lymphocytic reaction which appeared under these conditions was approximately of the same magnitude as the corresponding reaction to a single heterotransplantation.

EFFECT OF INJECTIONS OF TRYPAN BLUE ON THE CHANGES
IN LEUKOCYTE COUNTS FOLLOWING HOMOTRANSPLANTATION
AND HETEROTRANSPLANTATION

Trypan blue was injected over different periods, two, four and six days, respectively, preceding homotransplantation as well as heterotransplantation and again at intervals of two and four days immediately after transplantation, as well as at weekly intervals thereafter. Differential counts were made during the period of the preliminary injections, as well as every other day for a period of twenty days after transplantation. Guinea pigs served as hosts. They were divided into two groups of 6 animals each. The first was given injections of a 0.5 per cent solution of trypan blue in 0.5 cc. amounts; the second group, the same solution in 1 cc. amounts. Three animals in each group received homotransplants of thyroid gland. Three received heterotransplants of rat thyroid.

The preliminary injections of trypan blue as such in either dosage for periods of two, four or six days did not markedly increase the percentage of lymphocytes or of polymorphonuclear leukocytes. In 3 guinea pigs the lymphocytes increased slightly above 5 per cent. One showed an increase of as much as 8 per cent on one occasion. Generally, however, the results observed were similar to those previously described in normal controls, in which slight percentage deviations in a positive as well as in a negative direction occurred.

As a result of the transplantation, the lymphocytes rose generally to about 5 per cent, an effect which could be attributed to the operative interferences, and it was identical with that observed after autotransplantation or the implantation of inert substances. In two other experiments variations in leukocyte counts similar to those occurring in normal controls were seen. The changes characteristic of homotransplantation and heterotransplantation were prevented by the injections of trypan blue.

There were, however, three exceptions to these results, and all of these occurred in the animals receiving the smaller doses of trypan blue; 2 of these guinea pigs had received homografts, while the third one was the host of a heterotransplant. Moreover, 2 of these 3 animals had

been given injections of trypan blue only during a two day period; the third one had been given injections during a four day period preceding transplantation. These animals showed the usual reactions characteristic of transplantation of homogenous or heterogenous tissue, although in all of them the maximum increase in the number of leukocytes occurred rather late, namely, on the ninth day following the homotransplantations, and on the eighth day in the animal which had received a heterotransplant, an indication that even in these cases the reaction had been somewhat weakened.

When the transplants were removed from the host animals at autopsy, it was observed that the majority of them were surrounded by a blue peripheral ring. Whether the deposit of the dye in the periphery of the transplant prevented the diffusion of the organismal differential into the host, or whether the trypan blue prevented the reaction on the part of the leukocytes through a blockade of the reticuloendothelial system must be left undecided for the present.

EFFECT OF HEAT ON ORGANISMAL DIFFERENTIALS

In earlier investigations on tumors (Loeb^{2c}) and on amebocytes (Loeb and Drake^{22a}) it was shown that by exposure of tissues or cells to graded degrees of heat it was possible to induce a reduction of abnormalities in vital processes without causing actual death in the exposed cells and that under these conditions recovery of the impaired tissues might take place. Siebert²³ exposed pieces of normal tissues in vitro to higher temperatures before transplantation. Heating homogenous thyroid and cartilage at temperatures varying between 43 and 51 C. for thirty minutes caused a marked diminution in the strength of the lymphocytic reaction toward the homogenous graft, but it caused only a slight reduction in the reaction of the polymorphonuclear leukocytes and lymphocytes toward heterotransplants.

I studied the effect of heating tissues in vitro preceding transplantation on the subsequent action which homotransplants and heterotransplants exerted on the number and on the kinds of leukocytes in the circulating blood.

Various tissues were excised and subjected outside the body to different degrees of heat. After the tissue was removed from the animal, it was immersed in a sterile solution of 0.9 per cent sodium chloride which filled part of a test tube. The latter, containing the piece of tissue, was then placed for one-half hour in a water bath, previously adjusted to the desired temperature, which was kept constant. At the end of the period of heating, the tissue was removed from the test tube and either homotransplanted or heterotransplanted. Differential counts were made in the usual manner.

22a. Loeb, L., and Drake, D.: *J. M. Research* **44**:447, 1924.

23. Siebert, W. J.: *Arch. Path.* **12**:590, 1913.

Altogether, 69 experiments of this kind were carried out with homotransplanted and heterotransplanted tissues (table 14). The reactions were classified as typical if both the magnitude and the time of the maximum increase were within the limits established for the transplanted normal tissues. If the magnitude of the increase was about the same but the time of appearance later than normal, the reaction was designated as delayed. If the time of appearance was late but the maximum increase was only from 5 to 10 per cent, the reaction was termed weak, whereas if the increase was 5 per cent or less, the reaction was considered negative.

Effect on the Individuality Differential.—As can be seen from table 14, thyroid heated at 45 or 50 C. and then homotransplanted calls forth a normal or about normal reaction. There were 18 typical reactions, 2 were delayed, and in only a single experiment, that of homotransplantation of thyroid of the rat, did the guinea pig fail to show a reaction. Homotransplants of pigeon skin previously exposed to these two temperatures gave 5 typical reactions and 4 weak ones. However, the fact may be recalled that even normal, nonheated skin gives only a weak reaction after homotransplantation. These experiments suggest that heating at 45 or 50 C. may enhance the reaction elicited by skin to some extent, since the number of typical reactions observed under these conditions was greater than that following homotransplantation of the normal tissue. But further experiments will be necessary to make such a conclusion definite.

After being heated at 52 C., thyroid of the rat when homotransplanted called forth a typical reaction. Pigeon thyroid heated at 54 C. previous to homotransplantation induced typical reactions in 3 experiments, a weak reaction in 1 and no reaction in 2. Pigeon skin and thyroid and rat thyroid heated at 56 C. were inactive. The critical temperature in the case of homografted thyroid in pigeon seems thus to be approximately 54 C. Rat thyroid was not exposed to a temperature of 54 C., but since this tissue after being heated at 52 C. called forth normal reactions, whereas tissue previously heated at 56 C. failed to do so, it is probable that the critical temperatures for pigeon and rat thyroids are similar and that these tissues are inactivated at about 54 C. or at a slightly higher temperature.

Heating at 56 C. destroyed the action of guinea pig thyroid. However, guinea pig kidney heated at 56 C. for one-half hour and subsequently homotransplanted gave positive reactions in 2 of 4 cases; in 1 case the reaction was weak and in the fourth it was negative. These results indicate that in kidney tissue the homodifferential may be somewhat better protected against the injurious action of heat than in thyroid tissue.

In 8 experiments homotransplantation of pigeon thyroid was preceded by successive freezing and thawing. The thyroid gland was removed from a donor animal and immersed in sterile 0.9 per cent sodium

chloride solution in a test tube which was kept in an ice and salt mixture at 0 C. for one-half hour. The test tube containing the tissue was then kept at room temperature likewise for thirty minutes, when thawing took place. This process was repeated three times. Following the last thawing the tissue was homotransplanted. As can be seen in table 14, in 5 cases typical reactions were elicited, in 2 cases the reactions were delayed, and in 1 instance the reaction was weak. No negative reactions were observed. It thus appears that repeated freezing and thawing did not destroy the individuality differential but injured it to a slight extent.

Effect on the Species Differential.—Similar experiments were carried out with heterogenous tissues, but the latter were exposed to somewhat higher temperatures than homogenous tissues. Rat thyroid heated at 56 C. for one-half hour failed to give a reaction. The same negative result was obtained when guinea pig thyroid previously heated at 56 C. was transplanted to the rat. On the other hand, both kidney and cartilage, after having been heated at the same temperature, elicited the typical heteroreactions. Transplantation from rat to guinea pig of kidney previously heated at 60 C. for one-half hour gave 1 positive and 3 negative reactions, whereas in the reciprocal experiments no positive reactions were obtained. But with cartilage heated at 60 C. 50 per cent positive and 50 per cent negative reactions were noted; this, therefore, seems to be close to the critical temperature for this tissue, since after being heated at 65 C. cartilage failed to elicit a response.

From these experiments it may be concluded that the organismal differentials are destroyed at temperatures ranging approximately between 54 and 60 C., and furthermore, that the organismal differentials in denser tissues, such as cartilage, are somewhat more resistant to the injurious effect of heat than those in softer tissues, such as thyroid. Kidney stands midway between these two kinds of tissues as far as both density of structure and resistance to the injurious effects of heating are concerned. It needs still to be determined whether extraction of the organismal differentials occurs in these experiments and complicates their interpretation.

EFFECT OF TREATMENT OF TISSUES WITH CHEMICAL SUBSTANCES ON THE ORGANISMAL DIFFERENTIALS

In addition to studying the effect of heat on the organismal differentials I have begun an investigation of the action of various chemical substances on the homodifferential. So far it may be stated that solutions of 0.9 per cent sodium chloride in distilled water to which a few small crystals of thymol have been added in order to prevent the growth of bacteria seem to be relatively innocuous; but the large majority of the substances used proved very injurious, especially those which tended

to denature proteins, for such substances abolished the reactions to homodifferentials in the peripheral blood.

EFFECT OF PARENTERAL ADMINISTRATION OF PROTEINS, CARBOHYDRATES AND LIPOIDS ON THE DISTRIBUTION OF LEUKOCYTES IN PERIPHERAL BLOOD

As controls for the effects exerted by the different kinds of tissue transplants on the numbers of leukocytes circulating in the peripheral blood, a series of experiments was carried out in which different proteins,

TABLE 14.—*Effects of Heat on Organismal Differentials*

Animal Species	Tissue	Temperature, C.	Reaction			
			Typical	Delayed	Weak	Negative
1. Effect of Heat on the Individuality Differential *						
Pigeon	Thyroid	0	5	2	1	..
Pigeon	Thyroid	45	6
Pigeon	Skin	45	3	..	2	..
Rat	Thyroid	45	3	1
Pigeon	Thyroid	50	4	1
Pigeon	Skin	50	2	..	2	..
Rat	Thyroid	50	5	1
Rat	Thyroid	52	4
Pigeon	Thyroid	54	3	..	1	2
Pigeon	Thyroid	56	4
Pigeon	Skin	56	4
Rat	Thyroid	56	6
Guinea pig	Thyroid	56	3
Guinea pig	Kidney	56	2	..	1	1
2. Effect of Heat on the Species Differential †						
Rat to guinea pig	Cartilage	56	6
	Thyroid	56	3
Guinea pig to rat	Kidney	56	6
	Cartilage	56	4
	Thyroid	56	4
Rat to guinea pig	Kidney	60	1	3
	Cartilage	60	2	..	2	..
	Thyroid	60	2
Guinea pig to rat	Kidney	60	4
	Cartilage	60	2	2
	Thyroid	60	4
	Cartilage	65	6

* The total number of experiments was 69.

† The total number of experiments was 51.

carbohydrates and fatty substances were parenterally administered to guinea pigs and rats (table 15). These substances, whenever feasible, were implanted in a solid or semisolid state in order to reproduce as much as possible the mode of procedure used in the preceding experiments. In other cases liquid substances were injected. A plus in the table represents a positive reaction, which consisted in an increase in lymphocytes exceeding 12 per cent, occurring generally between the second and fourth days after the initial administration of the substance. This was the only positive response to the administration of certain of

these substances which took place, as will be discussed later. It corresponds to the reaction which is characteristic of homogenous tissues, as well as of homogenous blood and plasma clots, as far as the nature and magnitude of the reaction are concerned; as for the time at which the maximum of the increase in lymphocytes was reached, the reaction corresponds to that following homotransplantation of blood and plasma clots and brain, substances which are relatively soft, but not to that following homotransplantation of the large majority of tissues. However, the reaction differs in other important respects from that elicited by homogenous tissues.

Only the proteins were observed to induce this type of reaction. Gelatin implanted in the form of flakes had this effect. On the other hand, in the case of a single injection of a readily absorbable protein, such as fresh egg albumin, the reaction did not follow, owing, no doubt, to the great rapidity with which this substance is eliminated. When, however, egg albumin was injected on three successive days, the protein was retained sufficiently long to call forth the typical response on the part of the lymphocytes.

It is of special interest that neither partial denaturation of the albumin, caused by an addition of thymol crystals, nor complete denaturation, produced by an exposure to the temperature of boiling water, was followed by a loss of the ability of the protein to induce this reaction.

Casein, which is of animal origin, and edestin, a plant derivative, acted similarly to egg albumin.

These findings suggested tests with heterogenous blood serum. For this purpose use was made of rabbit serum, which was injected subcutaneously into rats or, after concentration, was implanted subcutaneously. Rabbit serum likewise did not induce a heterogenous reaction but a reaction similar to that seen after transplantation of homogenous tissues. There was a maximum increase in the number of lymphocytes, ranging from 15 to 25 per cent in the different experiments; the time of maximum reaction varied between the second and the fifth day. It may therefore be concluded that a mixture of rabbit serum albumin and globulin behaves in the same way as egg albumin and that it differs markedly in its effects from plasma clot, which acts like normal tissues. This difference in behavior between plasma clots and the corresponding blood serum is presumably due to the presence of fibrinogen in the former substance.

Of interest also is the fact that pieces of mouse embryo that had developed to a length of approximately 15 mm. did not, after transplantation in the rat elicit a heterogenous but a lymphocytic reaction characteristic of homogenous tissues; it is probable that the organismal differentials are not yet fully developed in these embryonal cells.

In addition to the differential counts of leukocytes, total counts were made on the peripheral blood of guinea pigs into which casein and edestin had been implanted. There was not only a relative increase in the number of lymphocytes but there was also an absolute increase comparable to the increase found in the differential counts following the administration of these substances. The absolute increase calculated from these data was also similar to that observed following homotransplantation, since in the 6 guinea pigs observed, 3 of which received casein and 3 edestin, this increase ranged between 58 and 81 per cent and averaged 69 per cent.

TABLE 15.—*Effect of Parenteral Administration of Proteins, Carbohydrates and Lipids on the Distribution of Leukocytes in the Peripheral Blood*

Substances	Animal Species	Method of Administration	Experiments	Reaction
Gelatin.....	Guinea pig	Implanted flakes	4	+
Fresh egg albumin.....	Guinea pig	Single injection	10	—
Fresh egg albumin.....	Guinea pig	3 successive injections	4	+
Fresh egg albumin and thymol...	Guinea pig	Single injection	6	+
Casein.....	Guinea pig	Implant paste	3	+
Edestin.....	Guinea pig	Implant paste	3	+
Fresh egg albumin.....	Rat	Single injection	4	—
Fresh egg albumin.....	Rat	3 successive injections	3	+
Fresh egg albumin and thymol...	Rat	Single injection	4	+
Heat coagulated albumin.....	Rat	Implant	4	+
Casein.....	Rat	Implant paste	4	+
Edestin.....	Rat	Implant paste	4	+
Sodium oleate.....	Rat	Implant	4	—
Sodium stearate.....	Rat	3 successive injections	4	—
Glycogen.....	Rat	3 successive injections	4	—
Saccharose.....	Rat	3 successive injections	4	—
Starch.....	Rat	Implant paste	4	—
Lecithin.....	Rat	Implant	4	—
Olive oil.....	Rat	3 successive injections	4	—
Normal rabbit serum.....	Rat	3 successive injections	4	+
Concentrated rabbit serum.....	Rat	Implant	4	+
Mouse embryo.....	Rat	Implant	4	+

All other substances used, carbohydrates as well as fatty substances, failed to induce a specific reaction; they induced merely the change characteristic of the operative procedures.

While the effect exerted by proteins on the number and kinds of leukocytes in the circulating blood is apparently the same as that elicited by homogenous tissue transplants, it differs from the latter in two important respects: (1) it is not prevented by exposure to a degree of heat which would completely destroy the action of all tissue, and (2) it is produced by material of heterogenous origin. It is therefore probable that one has to deal with a nonspecific or rather with a less specific reaction, characteristic of various proteins, which under the conditions in which they were administered in our experiments elicited only a mild disturbance in the organism, while in the majority of experiments of earlier investigators the strange proteins were injected intravenously

and under these conditions induced acute changes, becoming noticeable usually in the course of the first day after the injection. Schittenhelm and his collaborators ²⁴ and Wallbach ²⁵ observed, immediately following a single injection of such a substance, leukopenia, which in some of Wallbach's experiments lasted for only a few minutes; this was followed by leukocytosis, which had a duration of about six hours, after which the count returned to normal. A subsequent injection of the same material produced an anaphylactic reaction in the animal and, in addition, prolonged both phases, the leukopenia as well as the leukocytosis; under this condition the leukocytosis could be extended over a period of four to six days. Wallbach was able to produce such reactions with whole serum, but he observed that various fractions of the serum containing isolated proteins, such as albumin and globulin, were ineffective. The injection of bacteria was also followed by a first phase of leukopenia and a second phase of leukocytosis; Wallbach interpreted the prolongation of the leukopenic reaction as indicative of a lack of development of an active immunity to the injected micro-organisms.

On the other hand, Wiseman ²⁶ reported experiments in which embryonic chick extract, egg albumin or normal horse serum was repeatedly injected into animals either subcutaneously or intravenously. He found an increase in the total number of lymphocytes in the peripheral blood, varying from 23 to 139 per cent, which was noticeable from the seventh to the ninth day, in contrast to the reaction described by the aforementioned investigators, in which the leukocytes appeared on the first day. Furthermore, Wiseman's article contains no record of an abrupt rise in the animal's temperature accompanying the changes in the peripheral blood such as was noticed in the experiments of the other investigators.

It appears, therefore, that the changes which I have recorded are somewhat comparable to those observed by Wiseman but are different from those reported by Schittenhelm and Weichardt and also by Wallbach. The reactions which the latter workers observed were of a very acute nature, caused by intravenous injection of foreign proteins, a procedure which in a number of instances led to the death of the animal. The reactions of Wiseman and those which I have reported here, on the other hand, are of a milder character and due to a less active though long-lasting stimulation of the hemopoietic tissues.

24. Schittenhelm, A.; Weichardt, W., and Grissamer, W.: *Ztschr. f. exper. Path. u. Therap.* **10**:412, 1912. Schittenhelm, A., and Ströbel, H.: *ibid.* **11**:102 and 108, 1912. Schittenhelm, A., and Weichardt, W.: *ibid.* **14**:609, 1912.

25. Wallbach, G.: *Ztschr. f. d. ges. exper. Med.* **82**:22, 1932; *Folia haemat.* **58**:393, 1937.

26. Wiseman, B. K.: *J. Exper. Med.* **53**:599, 1931.

COMMENT

In the various chapters of the preceding report I have already discussed the principal results of these investigations. I shall now discuss the main conclusion which can be drawn from the experiments, and I shall try to correlate the data with those previously obtained by Loeb and his collaborators² in their study of the effects of organismal differentials on the local reaction in and around grafts.

The evidence presented in this paper proves that a generalized reaction characterized by both an absolute and a relative increase in lymphocytes in the peripheral blood follows homotransplantation and syngenesiotransplantation and that a corresponding increase in polymorphonuclear leukocytes follows heterotransplantation. These changes take place in addition to the localized changes in and around the transplant. Furthermore, in its main outlines the leukocyte response closely parallels the types of changes taking place locally. Thus, following homotransplantation and syngenesiotransplantation it is the lymphocytes which collect around the graft and then invade it, whereas the cellular changes about heterotransplanted tissue in the early stages consist largely in an accumulation of polymorphonuclear leukocytes.

Moreover, the two reactions, the general and the local one, coincide as far as time factors are concerned. Thus after homotransplantation of thyroid the lymphocytes begin the invasion of the transplant by the sixth or the seventh day, the approximate time at which the maximum increase in lymphocytes is observed in the peripheral blood. After heterotransplantation the changes described by Loeb^{2r} are of a more acute nature, leading to rapid destruction of tissue and involving, in the early stages, the polymorphonuclear cells rather than the lymphocytes. Correspondingly, here also the blood changes indicate a more acute reaction; the polymorphonuclear leukocytes are primarily increased, and they appear at an even earlier date than do the lymphocytes in the case of syngenesiotransplantation and homotransplantation, the maximum increase being reached on the fourth or the fifth day in the case of thyroid heterografts as compared with the maximum increase on the sixth or the seventh day following homotransplantation of the same kind of tissue.

The degree of increase of the leukocytes is also greater following heterografting than after homografting. In the case of thyroid there is more than 100 per cent increase following heterotransplantation, whereas there is only an approximate increase, varying between 50 and 75 per cent, following homotransplantation.

There is an additional similarity between the character of the local and that of the blood reaction. Loeb^{2r} showed that in the case of heterotransplantation a secondary invasion of lymphocytes occurs following the primary action of the polymorphonuclear leukocytes. In my experiments similar changes have been observed in the peripheral blood

reaction. The increase in polymorphonuclear cells is succeeded by a return to normal conditions, reached usually by the tenth day. But soon afterward there occurs an increase in lymphocytes, both absolute and relative, which reaches a maximum between the fourteenth and the sixteenth day, after which the count once more returns to normal.

The wide range in the intensity of the local reaction following syngenesiotransplantation, in which the changes may closely approach those following homotransplantation, on the one hand, and autotransplantation, on the other, is very similar to that seen in the blood cell reaction. Here in some cases of syngenesiotransplantation there occurs an early increase in the number of lymphocytes closely resembling a typical homoreaction, whereas in an instance representing the other extreme the maximum increase was not reached until the nineteenth day. On the average, both local and general syngenesioreactions are of a milder nature than homoreactions; a greater length of time is required until the threshold of effective stimulation is reached, and a longer period is necessary before the height of the reaction is attained.

Since the general reactions so closely parallel the local responses described by Loeb, it seems logical to assume that the mechanism involved is the same in both instances. One may therefore conclude that the transplants give off substances which correspond to the organismal differentials of the graft and that these substances diffuse not only into the area surrounding the transplant but also into the general circulation, where they exert a typical effect on the white blood cells, presumably by the intermediation of the various hemopoietic organs. In accordance with the relationship between the organismal differentials of host and transplant, these substances act as autogenous substances or as syngenesiotoxins, homotoxins and heterotoxins and thus produce their characteristic effect on the host cells.

SUMMARY

Following transplantation of various tissues, changes occurred in the absolute and relative numbers of the various types of leukocytes circulating in the blood which paralleled closely the reactions of leukocytes around the transplants. After homotransplantation this general response was characterized by an absolute and relative increase in lymphocytes; after syngenesiotransplantation the response was similar but, on the average, less intense and reached a maximum after a longer time. After heterotransplantation the primary reaction was of a different kind, being characterized by an early relative and absolute increase in polymorphonuclear leukocytes; but secondarily there occurred here, also, an increase in lymphocytes. By means of these reactions it could be shown that the lens of the eye possessed individuality and species differentials.

In experiments in which successive transplantations were carried out, a more rapid but less intense reaction followed the second transplantation, whether of homogenous or heterogenous tissue, than that which occurred after the primary grafting. The acceleration observed following the second grafting of tissues may have been due to the development of immune substances. If that interpretation should prove correct, it would not imply that the reactions elicited by a single transplant were also due to the formation of antibodies; on the contrary the very rapid onset of this reaction, even after the first transplantation, indicates that it was due to the diffusion of substances from the transplanted tissue into the body fluids. It was possible to prevent the reaction on the part of the circulating leukocytes by injecting trypan blue into the host animal preceding and following the transplantation of tissue. Under these circumstances this dye accumulated around the transplant; to determine whether it acted by obstructing diffusion of substances from the transplant into the host tissues or through blocking the reticulo-endothelial system needs further investigation.

In addition a study was made of the effects of temperature and of various chemical substances on the individuality and species differentials which are contained in the transplants, using the reactions of the leukocytes in the circulating blood as indicators. It was shown that the organismal differentials were destroyed by heating at temperatures varying approximately from 54 to 60 C. The exact temperature at which destruction took place was influenced by the density of the grafted tissue. Repeated freezing and thawing did not destroy the individuality differentials but may have caused slight injury in a few cases.

Of the chemical substances tested, only distilled water and 0.9 per cent sodium solution to which thymol had been added failed, as a rule, to destroy the individuality differentials. Isolated instances of non-destruction were observed with a few additional substances. All of the reagents and agencies employed which are known to cause changes in the constitution of proteins were found to be especially active in injuring the organismal differentials.

Whereas transplanted blood clots and plasma clots were able to elicit homoreactions and heteroreactions in accordance with the relationship between transplant and host, as shown by the changes in the distribution of the circulating leukocytes in the blood, injections of heterogenous blood serum failed to induce the heteroreaction. Similarly a number of protein substances of diverse origin failed to elicit the typical heteroreaction; instead, changes in the lymphocytes similar to those seen after homotransplantation occurred. Subjecting these substances to degrees of temperature which destroyed the organismal differentials of tissues did not, however, prevent these reactions. It is therefore probable

that in these cases the reactions were of a less specific nature than those induced by the organismal differentials. In contradistinction to protein substances, carbohydrates and lipoids did not in my experiments exert an effect on the number and distribution of the circulating leukocytes.

CONCLUSIONS

Transplanted pieces of tissues and organs give off substances which bear the organismal differentials and which not only diffuse into the areas of the host directly around the graft but, in addition, reach the general circulation; thus they may be carried to distant places, presumably to the leukocyte-producing organs, and there exert an effect on the number and kind of leukocytes which circulate in the blood. These substances act, therefore, not unlike hormones; it is probable that they are of a protein nature.

APPENDICITIS

I. CHANGES IN THE MUSCULATURE OF THE APPENDIX IN CHRONIC APPENDICITIS

JACQUES GOLDNER, M.D.

NEW YORK

This is the first article of a series dealing with the various clinico-anatomic forms of appendicitis. Others will appear later in the course of examination of the accumulated material, which is already considerable.

The study of the musculature of the diseased appendix has been neglected. To state, as do the authors of some comparatively recent reviews on the subject, that the muscular coats of the organ in chronic appendicitis are in certain instances thinned out and in others hypertrophied, and that the muscle cells are concomitantly degenerated, is to take too superficial a view and one that is not based on study devoted specially to the muscle and that is without the advantage of varied microscopic technic and finer histologic interpretation. Moreover, as I shall demonstrate, the thickness of the muscular coats, which varies extremely from one case to another, is in no way a cardinal sign. The musculature of the chronically inflamed appendix offers a multitude of indications which of themselves and independent of changes in the fibrous submucous capsule, lymphoid structures or epithelium permit one to make a definite diagnosis in the majority of instances.

While the aberrations in muscular structure to be reviewed here are characteristic of chronic appendicitis, they are not pathognomonic of it. One may meet with them in acute appendicitis, subacute appendicitis and all the subdivisions of these—even in normal appendixes. Nevertheless, the aberrations in structure are encountered more frequently in chronic appendicitis than in any other condition, their intensity is more striking and their diversity and variability are more marked. Nor should one overlook the fact that aberrant muscle fibers indicate a lesion in the organ to a more marked degree than do the fibrous or epithelial changes. The correlation between the structure of the smooth muscle and the past clinical history is more intimate than is that between the other coats and the symptoms if one excepts the perivascular reactions and the histiocytes.

The changes to be described are capable of furnishing an explanation of the genesis of the disease. Alterations in muscular contractions

From the Department of Surgical Pathology, Cornell University Medical College, and New York Hospital.

should, of necessity, correspond with the crossed fibers one finds, with the oblique fibers that are rare in normal controls, with the thickenings that constitute true sphincters and with the changes in morphologic arrangement of the coats. Should a spasmodic contraction of a certain intensity and duration arise in one of the areas of tangled muscle fibers or in a pseudosphincter, an entire segment of the organ, controlled by this nodal point, might find itself strangulated; micro-organisms would multiply, toxins be absorbed and cellulitis set up. This is, in short, verification of Dieulafoy's theory of the "closed vessel," not as regards the organ as a whole but rather as regards a segment thereof. That this is true is proved by the observation of certain appendixes in the series investigated which are unchanged in most of their length but show lesions in one segment. Another proof is afforded by the coexistence of muscular and epithelial, fibrous and lymphoid lesions. While muscular changes may exist independently, they are found to be most marked where lesions of the other coats are also most intense.

The lesions of these muscles reveal, at least in part, a very special form of histogenesis of smooth muscle. I have repeatedly confirmed this without, however, being able to find a pathogenic or a physiogenic explanation. A plausible interpretation, which cannot be applied in all cases, in which one demonstrates this histogenesis, is as follows: When one of the muscle coats becomes attenuated or fragmentary or fibrotic, the maintenance of function demands that new muscle fibers with the same orientation as those which have disappeared from the attenuated layer (usually the external longitudinal) should arise in its vicinity and reenforce it or become intercalated among those of the circular coat. It might be that some of these new-formed fibrils offset the abnormal contractions of the coat which has lost its function owing to a variety of lesions. Let me hasten to say that this is merely a hypothesis which cannot at the moment be verified.

The new formation of smooth muscle fibers, wherever situated, does not appear to be accomplished at the expense of preexistent musculature, as will be explained later. It seems rather to be the result of the stimulus of repeated, although abortive, attacks of appendicitis on the histiocytes, whether these are in the lymphoid or in the areolar tissue. This will be discussed at length. It is also important to decide whether these changes in the musculature are a result of such irritation or whether they are congenital. Only a statistical study could give a complete conception of the percentages of aberrant fibers in appendixes of all types and ages. However, aberrant fibers are exceptional in acute appendicitis, rare in the subacute form and frequently seen in chronic appendicitis. Were they already present from birth, one would expect

to find them in a finished, complete state in specimens showing appendicitis, but this is not the case—they are obviously in the process of formation.

The thesis of this work is that the newly formed muscle fibers, by reason of their arrangement and nature, cause rhythmic abnormal contractions of the organ that foster and prolong its pathologic symptoms. That they should be studied from this standpoint is the reason for the present contribution.

MATERIAL AND METHODS

This study is based on the examination of more than 100 appendixes removed in the department of surgery of the New York Hospital, of which about 60 showed chronic appendicitis of the generally recognized types. These have been compared with sections from appendixes showing acute and subacute (in the sense of continued acute) appendicitis and with others from normal appendixes removed incidentally in the course of other operations. Each specimen was received in a fresh condition, immediately placed in a refrigerator and examined about two hours after operation. For this work the apex, the second section taken proximal to it and all but one longitudinal strip were assigned to me, the one distal transverse segment and the single longitudinal strip being used for the departmental routine examination. Often, rolled preparations of the entire organ were prepared, affording sections that represented its entire length. After fixation the segments were sometimes cut into thinner slices and subjected to supplementary fixation.

The following fluids were used for fixation: a formaldehyde-alcohol solution (either 10 parts of solution of formaldehyde U. S. P. to 90 parts of 95 per cent alcohol or Schaffer's formula of 2 parts of from 80 to 95 per cent alcohol to 1 part of solution of formaldehyde U. S. P.); Bouin's solution prepared with trichloroacetic acid; Heidenhain's ^{1a} Susa fluid (mercury bichloride, saline solution, solution of formaldehyde and trichloroacetic and acetic acids), and Bouin-Susa fluid, which is the last-mentioned fixative saturated with trinitrophenol. Orth's, Zenker's, Helly's and Stieve's fluids were also used. The staining method of choice was my modification of the Masson trichrome light green technic.^{1b}

ALTERATIONS OF THE MUSCULATURE

The changes in the morphologic pattern of the muscular coats after repeated attacks of appendicitis are, of necessity, a response to inflammation. For this reason a large variety of pictures may be seen. While it might seem futile to attempt to describe these, a number of definite types of fiber groupings may be recognized and should be put on record for future reference.

Whorls.—The most frequently encountered form of whorl is characterized by fibers running in different directions in the same bundle. Some of these have the orientation one might expect in a given location, while others run obliquely or even spirally (fig. 1). Such whorls may abut on normal fascicles, but they are separated from their surroundings

1. (a) Romeis, B.: Taschenbuch der mikroskopischen Technik, Munich, R. Oldenbourg, 1932. (b) Goldner, J.: Am. J. Path. **14**:237, 1938.

by reticular or collagenous fibers. Often they are isolated by enveloping muscle fibers that course in a direction opposite to that proper to the fascicle. Originating in the interior of the whorl, they leave it in the form of wicklike bundles that proceed more or less to encapsulate it. They may join with similar bundles from other whorls to enmesh groups of whorls or to form a median, rather incomplete layer between two muscle coats (figs. 1 and 2). Their course after leaving the whorl may be devious.

These whorls may be grouped into masses (fig. 3), which may replace an entire region in one of the muscle coats, most often the

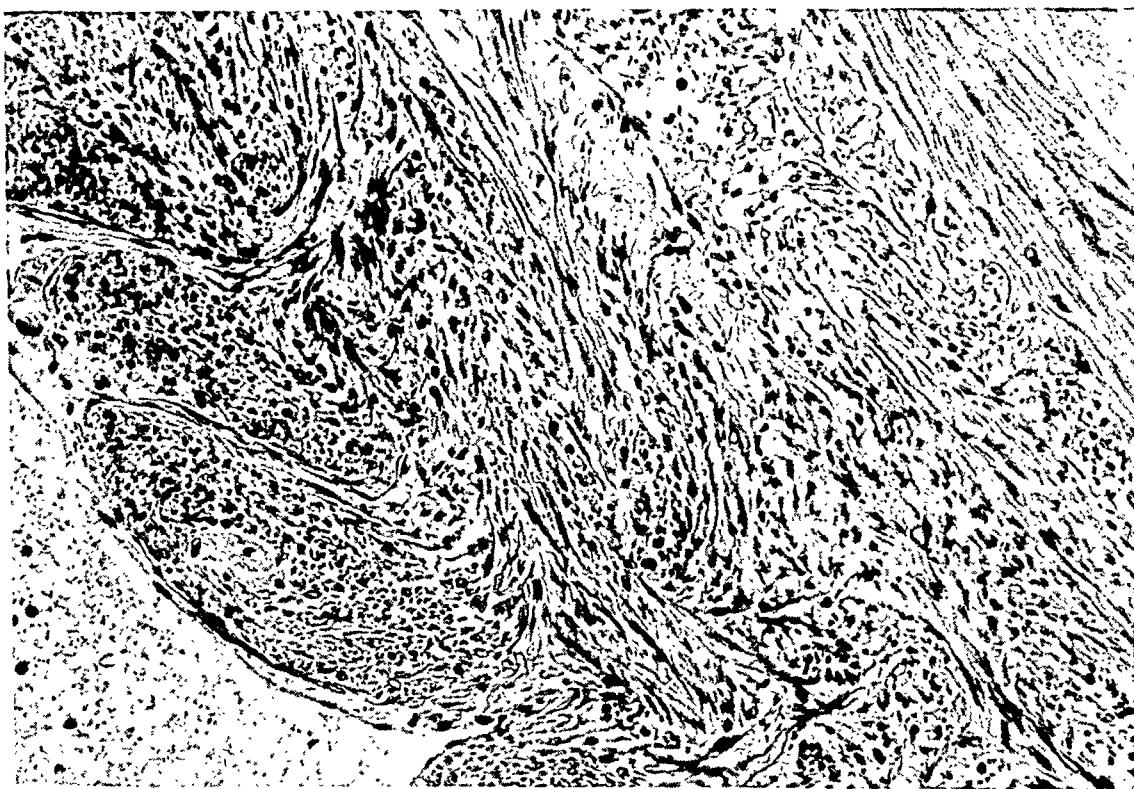


Fig. 1.—Chronic sclerotic appendicitis ab initio; transverse section. Note whorls, the coexistence of fibers running in different directions and enveloping fibers.

external, or may become intercalated between the two coats, forming a supplementary, or middle, coat. By their presence they recast the appearance of entire regions, whatever may be the histiologic makeup, and distort the normal topography.

Aberrant Fibers.—(a) *Mixed Fascicles*: These are usually situated in the external coat. Their dimensions are slender, and they are characterized by the coexistence within them of longitudinal and transverse fibers. Oblique and spiral forms are wanting. They do not appear to

produce penetrating fibers that leave the bundle to connect it with a layer running in the opposite direction. They rarely show enveloping fibers, which like the penetrating fibers are characteristic of the whorls. The mixed fascicle may be separated from similar bundles by connective tissue and tends to lie out of alignment, forced into a blind corner by neighboring nerve plexuses, vessels and the like. Everything indicates that it has an autonomous function.

(b) Mixed Fascicles with Interlacing Fibers: They are rarely seen. They occur only in the circular coat and may be so extensive as to occupy two low power fields. Their chief feature is their intricate weave.

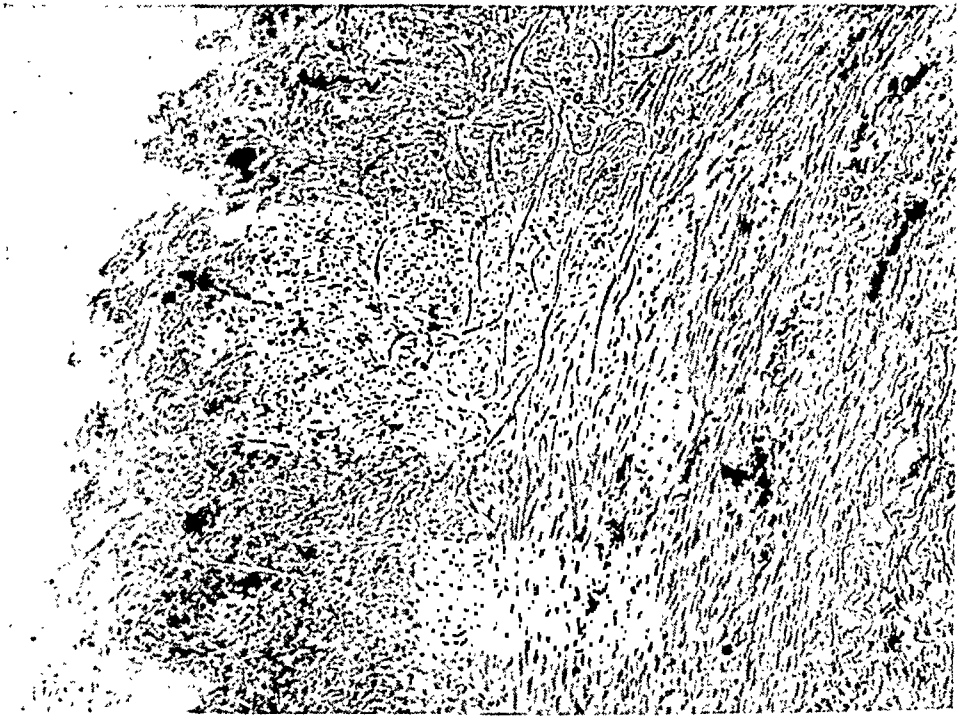


Fig. 2.—Chronic hyperplastic appendicitis ab initio; transverse section. There is a supernumerary middle layer between the external and the internal coat, formed by transversely running fascicles and surrounded more or less completely by circular or spiral fibers. The circular fibers trail off into narrow and isolated fascicles.

There are areas where fibers of one direction predominate over those running in another, in spite of their interlacing topography.

(c) Mixed Fascicles in Isolated Groups: These are characterized by their form, isolation and deeper staining affinities. They are usually situated in the circular coat and are most readily found in longitudinal sections. They are ovoid, and most of the fibers composing them run in the direction of the coat in which they lie. They tend to be coarser, to stain more deeply and to show a larger number of nuclei (fig. 4).

(d) Crosses: I have mentioned the "penetrating fibers" that leave the whorls to encase them or to mingle with similar fibers from other whorls; frequently they cross these, forming maltese crosses with a narrow point of intersection and flaring arms. The fibers may cross at an angle at the point of intersection or may present merely two loops that approach each other closely, with or without exchange of fibrils, to form a spencerian letter X (fig. 3).



Fig. 3.—Chronic ulcerative appendicitis; transverse section. A group of whorls may be seen. At the left, near the fat cells, are fibers penetrating into the fascicles of transverse fibers (external coat). Near the middle are three packets of transverse fibers enveloped by longitudinal fibers. Near the right are intersecting longitudinal fibers.

(e) Matted Fibers or "Herring Bones": These show fibers that intersect one another at acute angles and are more or less curved or even curled. The spaces left in this tangle are occupied by ganglion cells, nerves, capillaries and other structures; more important, there may be bundles of muscle fibers in them which run in curves.

(f) Bridge Fibers: These may be composed of a single fiber or of several, and broader or coarser ones may serve to join two muscle layers or portions thereof. They exist in large variety and have been noted arising from the subserosa, possibly reenforcing gaps in the external coat, but running counter to it. They may pass to the circular coat and where it has been cleft by vessels or other structures may be seen running across the cleftlike stays. Finally, they may unite the circular coat with the muscularis mucosae. They are sometimes seen to take a wavy, arabesque-like course and to occur multiply rather than singly; they may then be called arabesque fibers.



Fig. 4.—Chronic appendicitis; longitudinal section. There are mixed fascicles in isolated groups. Besides these deeply staining ovoid formations, note interlacing fibers.

(g) Loops: Fibers may arise in the circular coat, follow its course for a while after leaving it and then run out of the section. Serial sections, however, prove that after leaving the coat and describing a loop, they return to the circular coat. Another variety of loop is furnished by recurrent fibers that are a form of enveloping fibers, which leave their fascicle, the bundle increasing in thickness by reason of the addition of other fibers, and run obliquely or in curves to the internal coat, where they may or may not exchange a few fibrils (fig. 5). They then course back to the external coat and in so doing embrace whorls with multidirectional fibers, forming a true supernumerary layer of aberrant fibers.

(h) Penniform Fibers: These are another form of enveloping or penetrating fibers that are found in multiples rather than isolated. From each whorl in a given region, or from its capsule, fibers run obliquely toward a more or less isolated fascicle or toward the circular coat. This forms the shaft of the feather; the oblique fibers that join it, the barbs.

(i) Digitiform Expansions: These arise in the most central portion of the circular coat and are distributed through the submucous connective tissue, their destination usually being the muscularis mucosae. They are common in chronic appendicitis undergoing fibrous obliteration. They

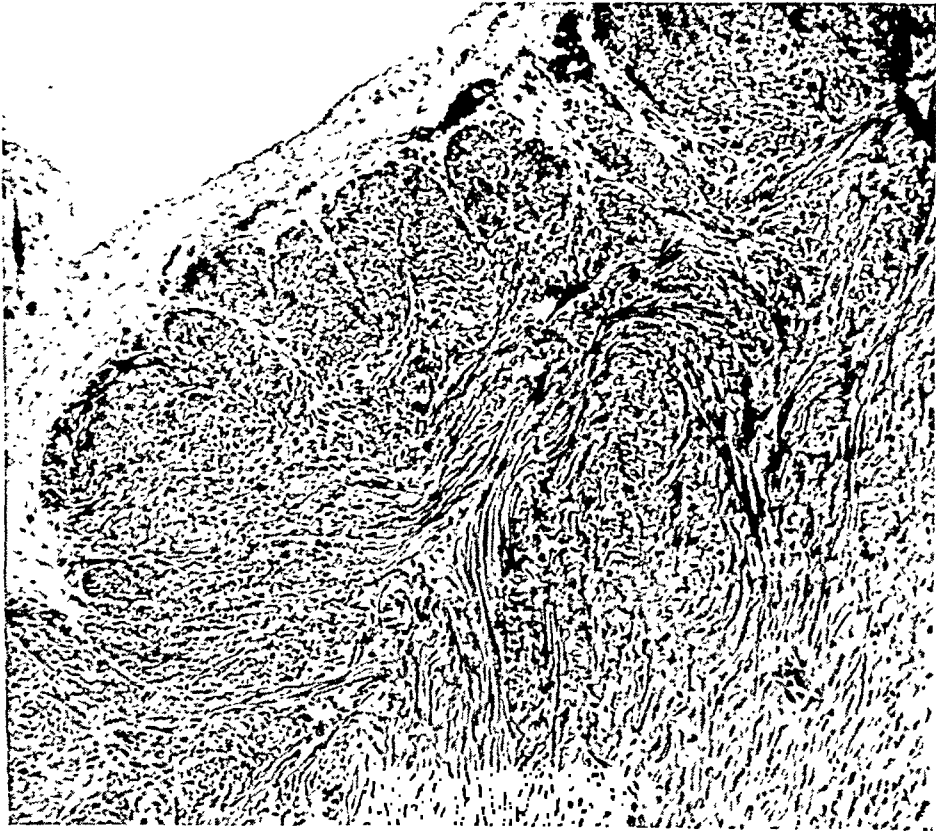


Fig. 5.—Chronic fibrous appendicitis; transverse section. Several groups of transverse fibers may be seen in the internal layer. Each packet is enveloped by longitudinal fibers, which reunite to outline these groups and then lose themselves after turning aside to the internal layer. Recurrent fibers are seen generally throughout the field.

run at first parallel to the circular coat, diverging obliquely and possibly decussating with other fibers; usually they form patterns like hands with outstretched fingers. Whorls may be formed in these digitations. The digitiform fibers may form blunt processes extending toward the muscularis mucosae without reaching it, or they may return to the fascicle whence they arose in the form of recurrent fibers. If they

encounter more or less rigid anatomic structures in their path, they may split to surround these, reuniting when the obstacle is passed.

Fate of Aberrant Fibers.—Of these the whorls, whorled fibers and supernumerary layers give one an impression of permanency; the other forms seem to be in a state of evolution and rearrangement. There appear to be processes of evolution going on in one part of them, while in another segment histolysis is taking place. Points of sarcolysis are, however, rare. The lytic processes are the work of histiocytes.²

CHANGES IN THE MORPHOLOGIC APPEARANCE OF THE MUSCULAR COATS PROPER

The muscular coats as such may show variations within themselves: whorls and aberrant fibers, changes in thickness and compensatory alterations.

Changes in Thickness.—The thickness of the muscular coats is extremely variable; the normal ratio between the external and the internal coat, maintained in acute appendicitis, is lost in the chronic condition. Most striking is the variability of thickness over long stretches, which may involve one or both coats. This is best noted in rolled sections of the entire organ, the longitudinal section giving more scope. Where one coat is thinned the other does not necessarily become thickened, as newly formed fibers that are strewn between the coats or in the submucous layer of connective tissue take care of compensation. In transverse sections there is sometimes noted a thinning in one semicircle of the organ which is counterbalanced, as it were, by a corresponding thickening in the same coat but in the other semicircle and on the opposite side of the organ. Numerical ratios vary tremendously; the inner coat may measure from 6 to 1, while the outer varies from 3 to 2.

Suppression of Coats; Occurrence of Supernumerary Coats.—The external coat may be fragmentarily wanting (fig. 6); it may be supplemented by arched or oblique aberrant fibers (fig. 7) or by connective tissue from the subserosa. When it is lacking, the internal coat is found to contain whorls, aberrant fibers or fibers that run in the same direction as those of the missing external coat. The transitions between the normal and the suppressed or aberrant region of a coat may occur abruptly or gradually. In the latter case, one sees successively fibers that run in all directions and form whorls; next, simpler packets of external coat and oblique fibers which trail off into subserous projections. At such points the external and internal coats become solidly fused, their fibers interlacing obliquely or at right angles.

2. Goldner, J.: Compt. rend. Soc. de biol. **144**:1131, 1933.

In other cases, the external coat instead of disappearing, appears to be formed of sparsely scattered fascicles of fibers. A majority of these have the appearance of fibers of the internal coat, running obliquely.

The supernumerary coat may involve either coat. The circular coat may be reenforced by increased connective tissue or by islets of muscle scattered through the submucous fibrous capsule, sometimes uniting the internal coat to the muscularis mucosae. The reenforcing fibers of the external coat may appear as islets with fibers running variously, astride the coat, scattered between it and the serosa or even invading

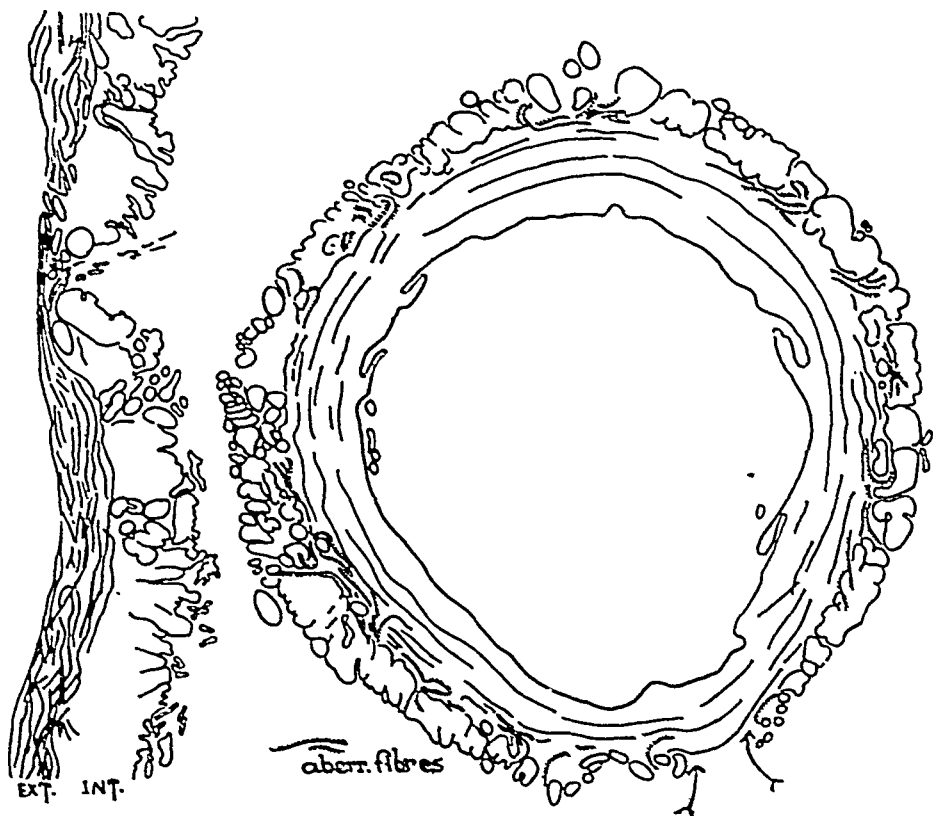


Fig. 6.—Diagram at left (chronic ulcerative appendicitis; longitudinal section): Note the variations in the thickness of the two coats. At one point the very thin external coat is reenforced by packets and fascicles from the internal coat. At this point digitations of longitudinal fibers run into the connective tissue. Notice also the intersections of the fibers of the external coat.

Diagram at right (chronic lymphoid appendicitis; transverse section): Note the variations in the thickness of the two coats. Between the two arrows one may note the disappearance of the external coat, which occurs gradually, stout packets being replaced by smaller and smaller fascicles. Note the geographic outline of the internal margin of the inner coat, the course of the aberrant fibers and the occasional transverse fibers that lie in the sublymphoid connective tissue.

the serosa. They may appear as isolated nodules which have nothing to do with the muscle itself, being completely separated from it. There are no bridge fibers here.

Another variation is intercalated fibers. Instead of showing two distinct layers, certain appendixes with chronic inflammation show first, an outer longitudinal or slightly oblique coat, a transverse coat, where the muscle bundles appear to be forced into the outer coat. This is succeeded by another longitudinal or slightly oblique coat and finally by the circular coat.

HISTOGENESIS OF ABERRANT MUSCLE FIBERS

I have never observed mitoses or amitotic division in the fibers of the two muscular coats or in the vascular cells, and classic myoblasts have been totally wanting. The only cell which could, then, produce an aberrant muscle fiber is the histiocyte, more particularly the adventi-

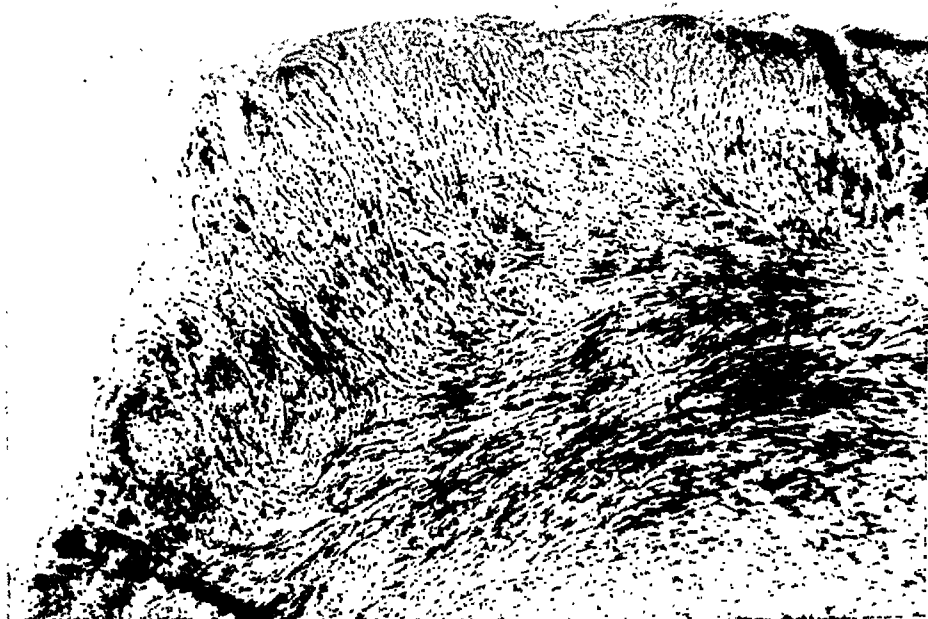


Fig. 7.—Chronic fibrous appendicitis; transverse section. The external coat is replaced by oblique fibers forming loops. The oblique fibers mingle with the circular fibers of the internal coat.

tial histiocyte of Marchand. In this respect the data presented here differ from those of Stieve,³ who believed that lymphocytes emigrated from the vessels of the pregnant uterus and became histiocytes which multiplied and produced muscle fibers, or that fibroblasts might accumulate early in pregnancy to produce muscle fibers later. He believed these were also of histiocytic origin. It is not evident in my sections that the lymphocytes (which are abundant) are ever transformed into muscle cells.

3. Stieve, H.: *Ztschr. f. mikr.-anat. Forsch.* **17**:371, 1929; *Zentralbl. f. Gynäk.* **53**:2706, 1929.

In drawing general conclusions as to this histogenesis one may point out:

1. The arrangement of newly formed muscle cells imitates that of newly formed capillaries (fig. 8). The capillary appears to form a sort of core for the production of muscle fibers; it may disappear, for many such vessels are merely temporary in function, and their development may remain uncompleted (e. g., there may be absence of an endothelial



Fig. 8.—Newly formed muscle fibers the arrangement of which suggests that of building capillaries.

lining). Their temporary morphogenesis seems to afford a form of preparation for the histogenesis of muscle fibers.

2. New muscle bundles, like new vessels, employ the interstices of the connective tissue as formative molds.

3. Muscle fibers may pass from one capillary to another, sometimes constricting them and causing them to disintegrate and cease to function (fig. 9).

4. The new formation of muscles stimulates proliferation of reticulum, reticular fibers determining the direction of the muscle fibers and separating them from each other (fig. 10 *A, B, C* and *D*).

5. The earliest muscle fibers appear as sleeves around the capillaries; by the time that they have been formed there are no adventitial cells left, so that no more muscle can be produced. These muscle cells do not divide; they merely grow in size. Fibers connecting them with other groups are produced by histiocytes in the intervening reticular layer (fig. 10 *E*).

It has not been possible to follow every stage in the transformation indicated in the foregoing paragraphs, nevertheless one may deduce that



Fig. 9.—Capillaries undergoing evolution. At the right, a capillary has disappeared (neither endothelium nor perithelium being visible); sparse muscle fibers envelop it partially. In the capillary that runs longitudinally (below) there are endothelial and perithelial reactions. One of the histiocytes has produced fibrils. The other is elongated into a spindle; its cytoplasm is dense and darkly staining. The three capillaries that run transversely are compressed by the smooth muscle fibers that course among them. The adventitial cells show fibrils. The neighboring histiocytes, increased in number, are of the reacting type, some of them showing fibrils. Supporting fibers connect transverse with longitudinal capillaries.

the disintegration of capillaries sets free the adventitial cells, which become more like Maximow's polyblasts as the process goes on, and that these cells may begin producing myofibrils even before they have assumed a fusiform character (fig. 9). These phases can be seen in my sections.

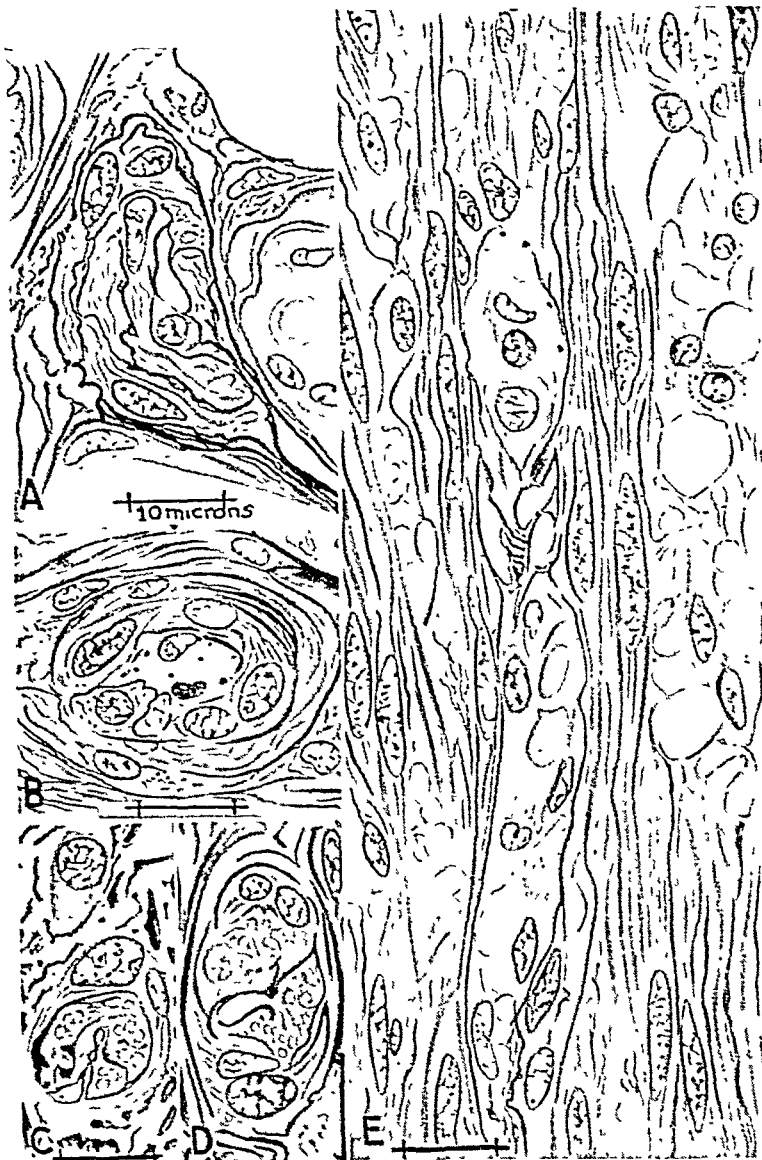


Fig. 10.—Capillaries at the time of formation of smooth muscle fibers: In *A* note the narrowed lumen, the metamorphosing endothelial cell and the altered perithelial cells containing fibrils. Reticulum separates the cells from one another. In *B* note the narrowed lumen, the fragments of erythrocytes, the desquamating endothelial cells, the outlining of the lumen by reticulum and the histiocytes with a fibrillary structure. In two of the latter the fibers run transversely, while those in neighboring cells run in the opposite direction. *C* shows an obliterated lumen bordered by reticulum. The muscular fibers run transversely. The fascicle is enveloped by smooth muscle fibers that emanate from the perithelial cells. The evolution of one of the perithelial cells is retarded (plump nucleus, rare fibrils, a reacting type). *D* shows disappearance of the endothelium, with effacement of the lumen. The reticulum that surrounds it joins that which separates the muscle fibers from one another. Smooth muscle fibers run in two directions. Note the evolutionary phase of the adventitial cells. Nearby is a plasma cell. *E* shows capillaries running longitudinally. Note the metamorphosis of the endothelium (desquamation; monocytic and polyblastic appearance). The endothelial tube is ensheathed by muscle cells through a transformation of the perithelial tube. Some adventitial cells of the reacting type are seen below. A second file of muscle fibers is apposed (at right); their origin is histiocytic. Note the reacting phase of the histiocytes of the reticular network. In this figure the scales shown are all drawn to 10 microns.

If one follows the direction of the myofibrils contained in these adventitial cells in the course of transformation, one finds them running longitudinally in some, transversely in others, in their immediate vicinity (fig. 10 *B*). Thus, the muscular sheaths of some capillaries are composed of fibrils running in two opposed directions. Part of these fibrils surround the vessel as long as it is functional; the more peripherally situated cells, however, leave the vessel and push out along its longitudinal axis (fig. 10 *E*). The new muscle fibers may remain more or less as they are, or they may increase in number and form bundles, the increase being the result of a transformation of histiocytes, which also form reticulum and direct the course of the muscle fibers, rather than of a proliferation from adventitial cells.

Fibers arising from capillaries that run at angles to the normal muscular coats deviate from the vascular axis at once and retain this deviation. Instead of reenforcing one of the coats, they become aberrant fibers of the bridge or loop type. Their direction is first dependent on that of the capillary; later it is determined by the tissue spaces, where the capaciousness allows the formation of arabesques and where the contractions of the altered muscular coats may force the aberrant fibers into new courses.

Muscular fibers are not formed alone at the expense of the capillaries that course in the muscular coats; they are also derived from those that lie in the connective tissue separating these coats from each other or from the submucous fibrous capsule. In the latter instance, the fibers that arise constitute the aberrant musculature that forms the median intercalated coat, digitations of which join the muscularis mucosae or whorls and groups of fibers that represent the displaced remains of attenuated anatomic coats that are in regression.

CONCLUSIONS

In chronically inflamed appendixes there are morphologic deviations of the two muscular layers, with attenuation or thickening, disappearance and the production of supplementary fibers.

Aberrant fibers appear ectopically, forming median coats, surrounding lymph follicles or lying in the fibrous capsule of the submucosa.

The structural changes described here are frequently seen in chronic appendicitis, fairly frequently in the subacute inflammation that signalizes early chronic appendicitis and very rarely in acute appendicitis and in normal conditions of the appendix. Their presence denotes past attacks that have stimulated the production of capillaries and histiocytes. They exist even when other cardinal signs (notably those proper to the lymphoid apparatus and epithelium) have subsided.

Functional changes in the form of abnormal contraction should correspond to these structural abnormalities of the musculature, which would in part explain the pathogenic mechanism of this disease. Abnormal contraction may be as important etiologically as changes in the rigid connective tissue framework, which also shows lesions of past attacks.

Aberrant fibers, as well as those that cause the thickening of one or the other coat, have a special histogenesis; their parent cell is the histiocyte, more particularly the adventitial cell of Marchand.

Preformed or newly formed capillaries disappear.

Although the majority of aberrant muscular structures appear to be permanent, others may fall prey to sarcolysis or to rearrangement. The lytic forces involved are supplied by histiocytes.

Case Reports

RÉTICULOENDOTHELIOMATOSIS

DOUGLAS SYMMERS, M.D.

General Director of Laboratories, Department of Hospitals, City of New York;
Professor of Pathology, New York University College of Medicine

AND

WILLIAM HUTCHESON, M.D.

Assistant Pathologist, Bellevue Hospital; Instructor in Pathology,
New York University College of Medicine

NEW YORK

In the Laboratories of Pathology at Bellevue Hospital we have had occasion to study a neoplastic disease involving not only those organs entering into the formation of the reticuloendothelial system—the spleen, lymph nodes, bone marrow and liver—but also structures not customarily regarded as members of that system, among them, the pericardium and pleura, both adrenals, the pancreas, the small intestine, the gallbladder, both kidneys, the uterus, fallopian tubes and ovaries, and the thyroid. The individual tumors are characterized histologically by an overgrowth of minute channels lined by endothelial cells provided with delicate spine-like processes which are impregnable by silver. The spleen was massive, and the lymph nodes were enlarged to a remarkable degree. The bone marrow was replaced over a wide expanse by tumor growth, and the patient presented the picture of a variety of myelophthitic anemia. We were unable to find a description of any comparable condition in the literature of medicine, and the disease is believed to be unique. It is best designated, we think, as reticuloendotheliomatosis.

REPORT OF A CASE

A white woman aged 42 was admitted to Bellevue Hospital to the medical service of Dr. Charles E. Nammack, Nov. 6, 1933, and died March 5, 1934. She complained that for eight months she had suffered from constant generalized pains referable to the bony system. She described the pains as "drilling." She stated that two months prior to examination she had noticed masses in both armpits and a tender mass in the upper part of the abdomen on the left side. She said that she had lost 40 pounds (18 Kg.) in the past year, and she complained of shortness of breath.

The patient was obese. The skin was flabby and presented a diffuse lemon yellow tint. Petechiae and small ecchymotic extravasations were visible in the skin covering both arms and legs. The cervical, axillary and inguinal lymph nodes were enlarged. The spleen was palpable at the crest of the left ilium and was tender. The edge of the liver was felt 6 cm. below the level of the costal slope in the right midmamillary line. The organ was tender. Palpation elicited tenderness throughout the bony system. Both legs were markedly edematous. The patient was dyspneic. The temperature ranged between 99 and 100 F.

From the Laboratories of Pathology, Bellevue Hospital.

Blood counts made over a period of several months averaged 1,500,000 red cells. The hemoglobin content ranged from 25 to 30 per cent. The white cells numbered on the average 20,800 per cubic millimeter, with a high percentage (82) of polymorphonuclear neutrophils. There were many normoblasts, megalo-

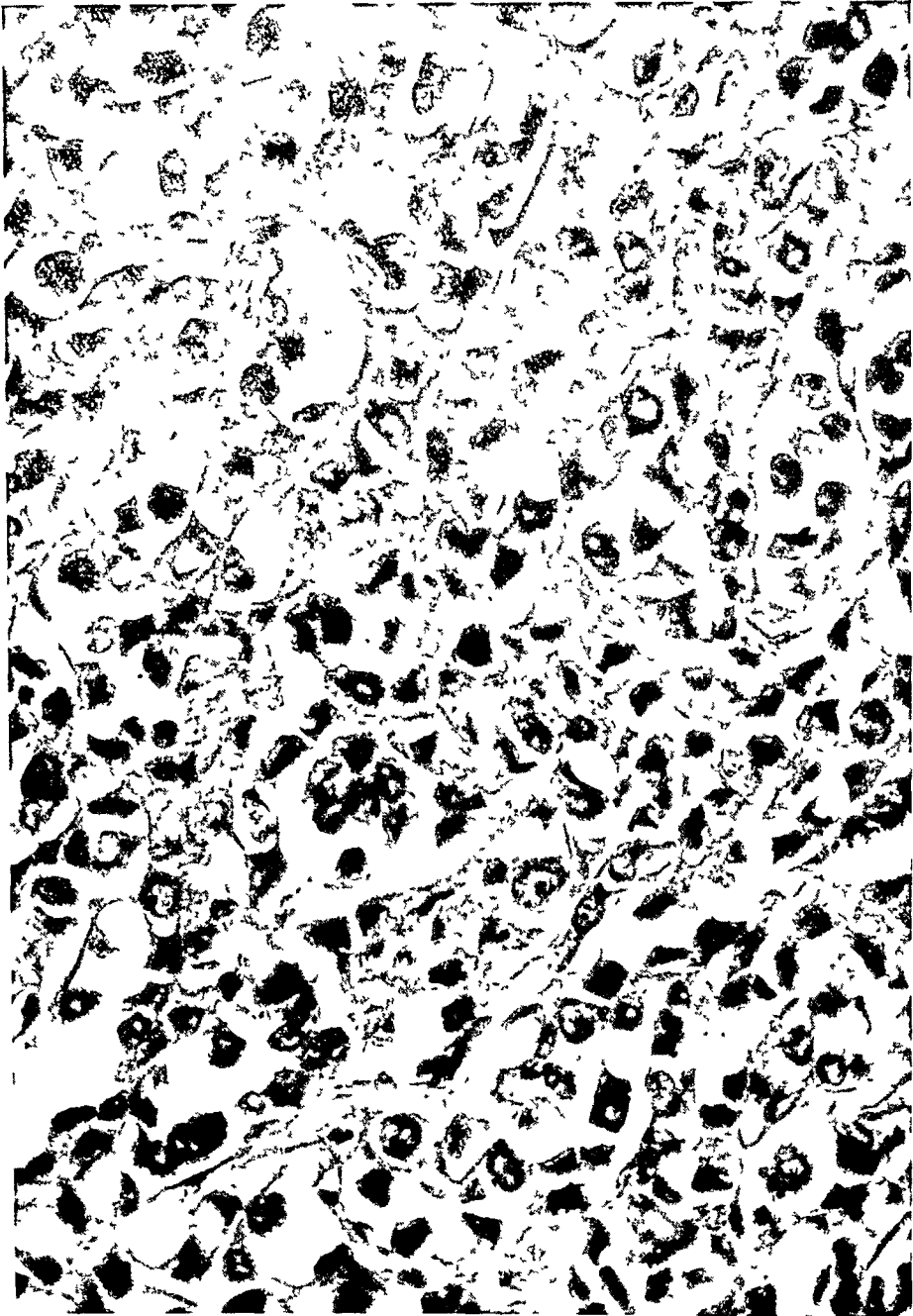


Fig. 1.—High power photomicrograph of a lymph node, showing minute channels lined by reticulum cells with spinelike processes; silver impregnation

blasts and macrocytes, a few reticulocytes, some anisocytosis and an occasional myelocyte. The color index varied from 1 to 1.13. The platelet count averaged

14,430; the coagulation time, one and a half minutes; the bleeding time, five and a half minutes. The icterus index was 5. The urine on repeated examination was normal.

Roentgen examination showed rarefaction and sclerosis in the rami of the pubic bones, in the ilia and in the upper third of the femurs, rarefaction of the skull and sclerosis of the lumbar region of the spinal column. The changes were interpreted as those of a metastatic malignant tumor, probably carcinoma.



Fig. 2.—Low power photomicrograph of spleen, showing interlacing reticulum fibers dividing the pulp into small islands; silver impregnation.

Microscopic examination of an excised inguinal lymph node showed changes which were interpreted as those of a metastatic adenocarcinoma.

Necropsy.—The incision was limited from the xiphoid cartilage to the pubic symphysis.

The body was that of an obese white woman 155 cm. in height and weighing 102 Kg. There was marked edema of the lower extremities, the left hand and

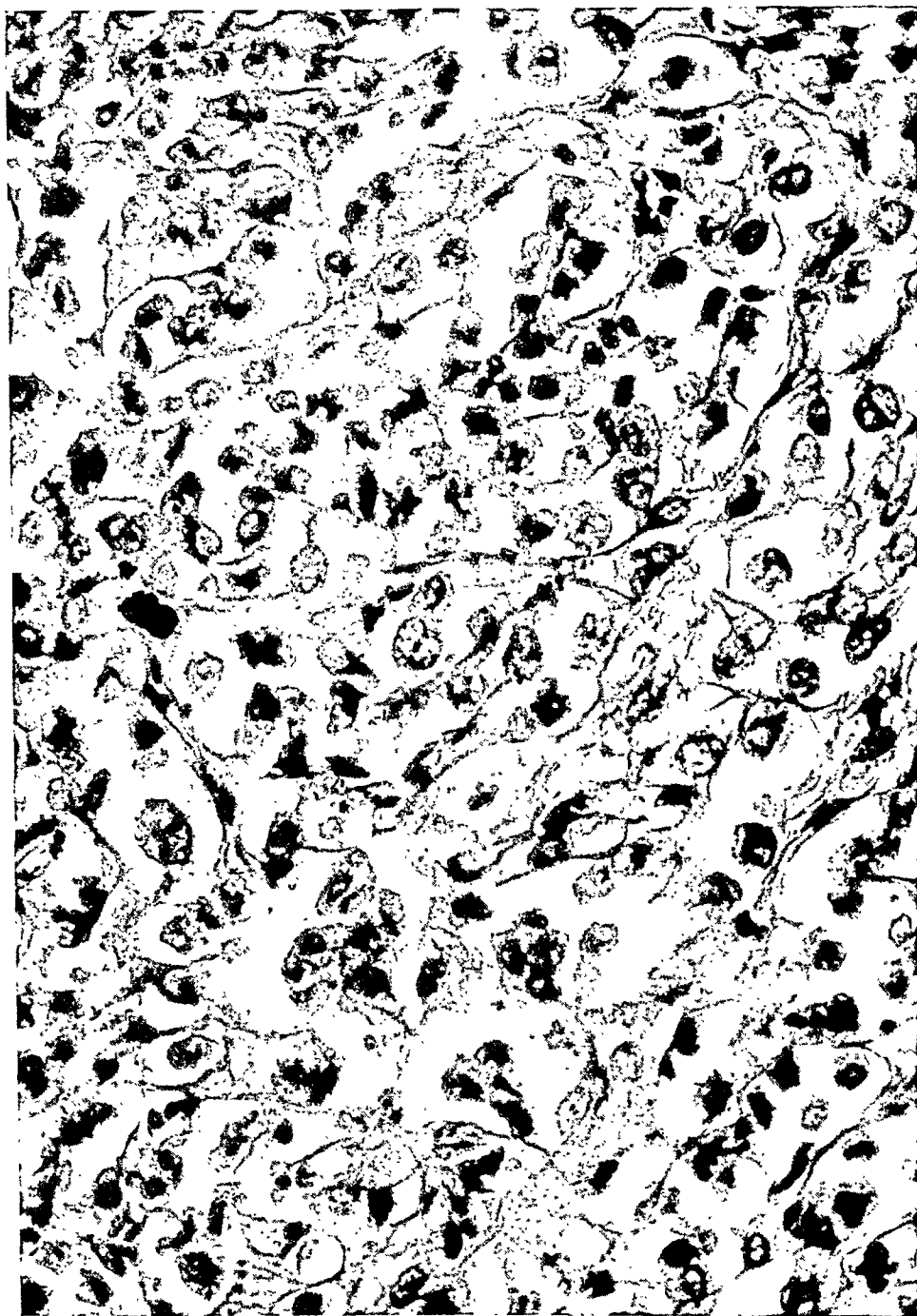


Fig. 3.—High power photomicrograph of spleen, showing minute channels lined by reticulum cells with spinelike processes; silver impregnation. Compare with figure 1.

the region of the right breast. The fat in the anterior abdominal wall was abundant; the muscle tissues were atrophic. The peritoneum was smooth, and the sac contained about 2,000 cc. of thin straw-colored fluid. Embedded in the pericardium, which for the greater part was smooth and glistening, were numbers of grayish dome-shaped nodules, sharply demarcated, sessile and from 1 to 3 mm. in diameter. The pleura of both lungs revealed extensive smooth grayish opaque

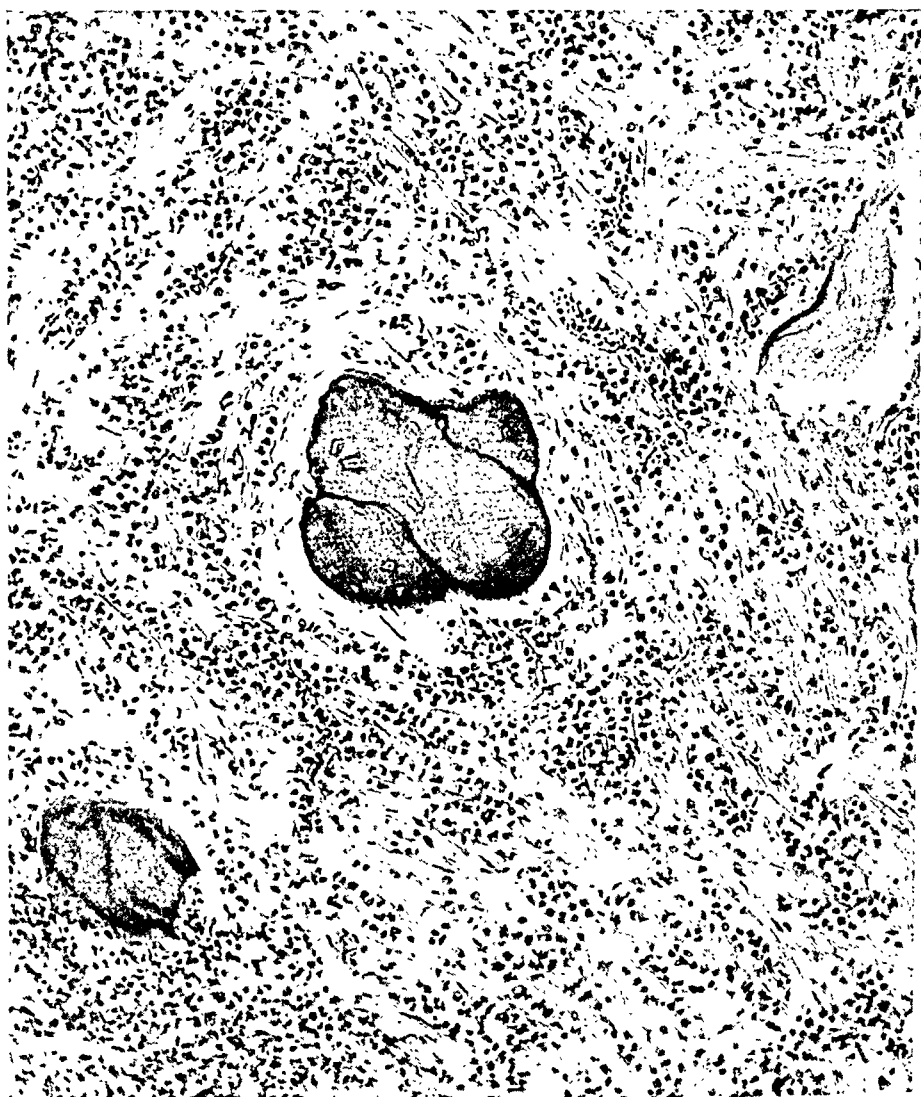


Fig. 4.—Low power photomicrograph of bone marrow, showing the formation of delicate canals and the abundance of intercanalicular substance; hematoxylin and eosin.

areas of thickening and numerous nodules of the same size and character as those described in the pericardium, together with delicate grayish linear projections into the substance of the membrane. The spleen weighed 1,150 Gm. The organ was red and tough, the capsule smooth. On section the substance was riddled with ill defined opaque grayish foci, measuring from 3 to 6 mm. in diameter. The liver weighed 3,075 Gm. Scattered through the capsule were moderate numbers of grayish, dome-shaped nodules from 2 to 15 mm. in diameter. Strewn through the substance were nodules of the same character as those described

in the capsule. The walls of the gallbladder contained a few nodules of similar appearance, averaging about 6 mm. in diameter. The pancreas also showed moderate numbers of nodules of much the same size and appearance. The adrenals were greatly increased in size and riddled with poorly defined grayish nodules varying in size from 1 to 4 cm. Scattered through both kidneys were a few nodules, measuring about 2 mm. in diameter and differing in no essential from

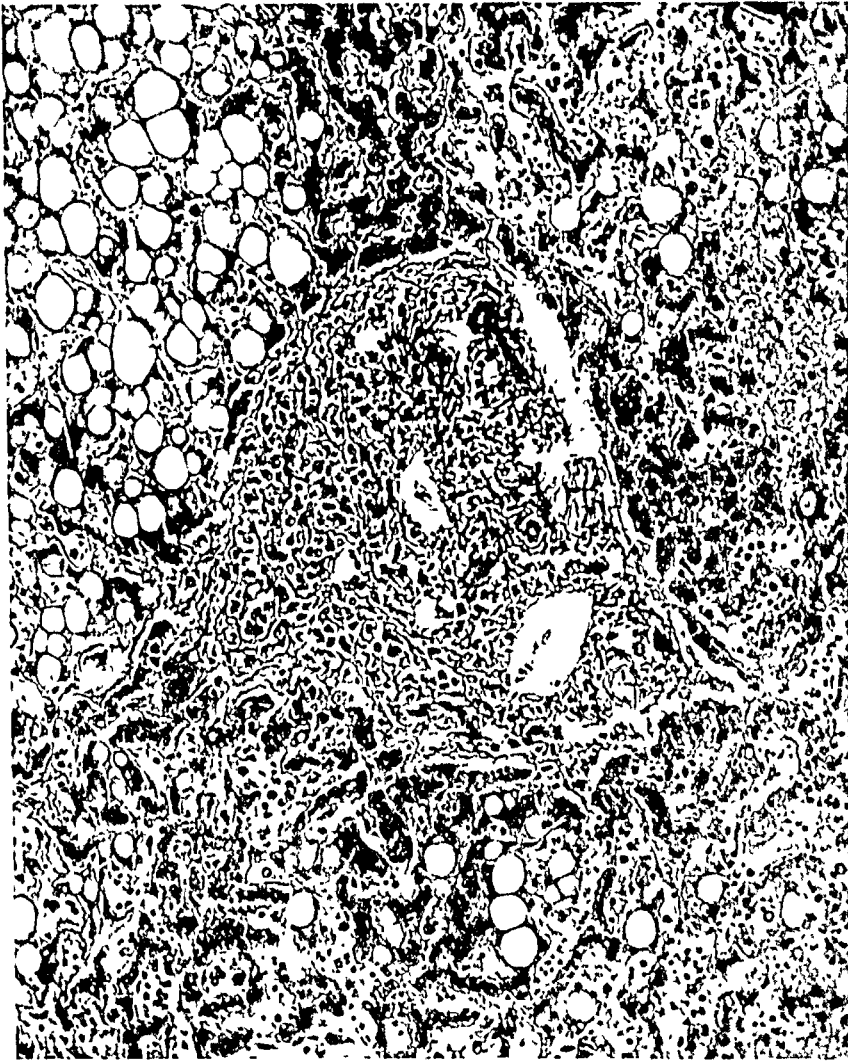


Fig. 5.—Low power photomicrograph of liver, showing atrophic and fatty parenchymal cells and interlobular collection of minute channels lined by reticulum cells; silver impregnation.

those encountered elsewhere. Embedded in each ovary was a solitary nodule, 1 cm. in diameter, of the same description as those referred to. The walls of the fallopian tubes were thickly strewn with nodules, all about 2 mm. in diameter. Two subserous sessile nodules, measuring, respectively, 3 and 8 mm. in diameter, were present in the fundus of the uterus, and several nodules, about 4 mm. in diameter, were buried in the endometrium. The cervical, peribronchial, anterior mediastinal, mesenteric and pelvic lymph nodes were enlarged, varying in diameter from 1 to 3 cm. The retroperitoneal nodes were extensively involved, and those

in the celiac region formed a mass 10 cm. in diameter. The cut surfaces of the lymph nodes presented a grayish opaque homogeneous appearance with faint undulations. The marrow of the pubic bones, lumbar vertebrae and ribs was practically completely replaced by firm grayish opaque tissue. In the upper part



Fig. 6.—High power view of a part of the area shown in figure 5. Note atrophic and fatty parenchymal cells and minute channels lined by reticulum cells with spinelike processes; silver impregnation. A few reticulum fibers are present.

of the right lobe of the thyroid were two firm grayish nodules, each measuring about 1 cm. in diameter. Permission for examination of the head was not obtained. Other than the changes described, necropsy revealed nothing worthy of record in the present connection.

Histologic Observations.—Lymph Nodes: These were partially or completely replaced by an overgrowth of capillary channels, limited externally by threadlike membranes, lining which were continuous or broken layers of irregularly shaped cells containing scanty, slightly acidophilic cytoplasm with delicate spinelike processes. These cytoplasmic processes were not infrequently to be seen in sec-

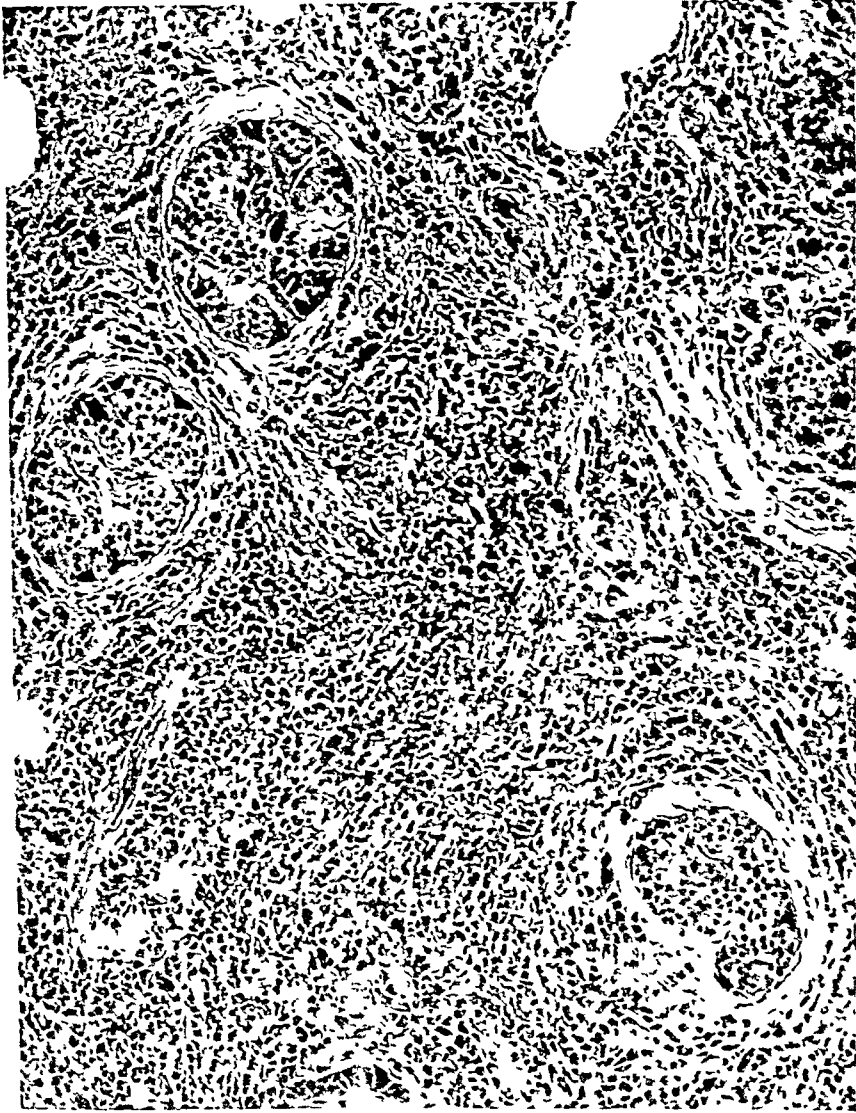


Fig. 7.—Low power photomicrograph of pancreas, showing three islands of Langerhans surrounded by reticulum cells arranged concentrically or in linear formation; hematoxylin and eosin.

tions stained with hematoxylin and eosin but were accentuated when impregnated with silver (fig. 1). In some instances the capillary growths extended into the capsule and perinodal fat tissues. In many instances the capillary channels were partially blocked or even packed to the point of distention by detached cells identical in morphologic character and staining characteristics with those just described.

Spleen: The architecture was practically completely obscured by an overgrowth of pinkish-staining, poorly nucleated tissue, which divided the substance of the organ into islands of different shapes and sizes and of variable cell content. In sections impregnated with silver (fig. 2) this supporting tissue was seen to consist of a dense network of reticulum fibers. In places it surrounded islands of pulp and sent individual reticulum fibers into the pulp. In other places were islands made up of capillary vessels lined by argentophilic endothelium with spinelike processes. These cells were scarcely distinguishable from the tumor cells in the lymph nodes (fig. 3). In some places the hyalinized walls of an arteriole were visible, and the insular structure as a whole was thus recognizable as an altered lymph follicle.

Bone Marrow: Many of the interstices in the bone marrow were filled with capillary channels lined by endothelial cells with argentophilic cytoplasmic processes. These cells were of the same appearance as the tumor cells in the lymph nodes and spleen (fig. 4). In silver preparations the intercanalicular tissue was shown to consist of interlacing reticulum fibers. In other interstices were closely crowded marrow cells whose identities could not always be distinguished in paraffin sections, although eosinophilic myelocytes were abundant and megakaryocytes were occasionally discernible.

Liver: The lobular formation was lost as was the columnar arrangement of the parenchymal cells. The latter were distorted and atrophic to an extreme degree, and many of them were coarsely vacuolated (fat). The sinusoids appeared to be greatly widened. The Kupffer cells were unchanged. In the perilobular connective tissues were numerous collections of capillary vessels which in sections stained with hematoxylin and eosin and in sections impregnated with silver were apparently identical with those in the lymph nodes, spleen and bone marrow (fig. 5). The foci in question were well circumscribed and were often irregular or spider-like in contour, the capillary vessels extending for short distances between immediately adjacent liver cells (fig. 6). Such foci varied in size from islands of microscopic dimensions to those which replaced one or many lobules.

Pericardium, Adrenals, Kidneys, Small Intestine, Pancreas and Uterus, Including Myometrium and Endometrium: The histologic picture in all these tissues was essentially identical with that in the lymph nodes, spleen, bone marrow and liver, but all of the former organs revealed in addition to the overgrowth of capillary vessels lined by endothelial reticulum cells the same sort of reticulum cells distributed in solid rows running parallel with one another or arranged concentrically (fig. 7).

COMMENT

It is our impression that the neoplastic disease described in this paper under the designation of reticuloendotheliomatosis is unique and that it is a clinical and pathologic entity. It is characterized (1) by multiple small nodules in various organs, including not only those of the reticulo-endothelial apparatus but also the pericardium and pleura, both adrenals, the pancreas, the small intestine, the gallbladder, both kidneys, the uterus, fallopian tubes and ovaries, and the thyroid, (2) by growth units consisting of minute channels lined by argentophilic endothelial cells with spinelike processes, and (3) by a variety of myelophthisic anemia due to widespread neoplastic replacement of the bone marrow.

HEMANGIOENDOTHELIAL SARCOMA OF THE THYROID WITH EXTENSION INTO THE TRACHEA AND WITH MASSIVE HEMOPTYSIS

MORRIS A. SIMON, M.D.,* MONTREAL, CANADA

Hemangioendothelial sarcoma is an uncommon tumor of the thyroid. At the present time the literature reveals reports of 21 cases, only 1 of which was published in America. The condition is not so infrequent as the figure stated would indicate, for Wegelin¹ noted the appearance of 19 cases of this type of tumor in his material at Bern, Switzerland, during the period from 1907 to 1922. A further review of the material at Bern (from 1922 to 1938) by me reveals 22 additional instances. The cases reported are distributed as follows: Hedinger,² 6 cases; Limacher,³ Frattin⁴ and Clivio,⁵ 2 cases each, and Usui,⁶ Winnen,⁷ Matti,⁸ Blum,⁹ Distefano,¹⁰ Wülfing,¹¹ Kassel,¹² Ponhold¹³ and Rice,¹⁴ 1 case each.

As the name indicates, the tumor is believed to arise from proliferating endothelium of blood vessels, and in its growth it simulates the formation of small vessels and capillaries. The morphologic appearance of the cells comprising the tumor also points to endothelial derivation.

From the Pathologisches Institut, Berne, Switzerland, Prof. Dr. C. Wegelin, director.

*E. E. Wolf Fellow in Pathology, Institute of Pathology, Western Reserve University, Cleveland.

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2. Hedinger, E.: *Zur Lehre der Struma sarcomatosa*. I, Frankfurt. *Ztschr. f. Path.* **3**:487, 1909.

3. Limacher, F.: *Ueber Blutgefässendothelien der Struma, mit einem Anhang über Knochenmetastasen der Struma maligna*, Dissert., Bern, Berlin, 1898.

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5. Clivio, R.: *Pathologica* **14**:759, 1922.

6. Usui, T.: *Berl. klin. Wchnschr.* **44**:1975, 1911.

7. Winnen, P.: *Frankfurt. Ztschr. f. Path.* **23**:405, 1920.

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11. Wülfing, H. W.: *Ztschr. f. Krebsforsch.* **41**:74, 1934.

12. Kassel, V.: *Die bösartigen Geschwülste der Schilddrüse*, Inaug. Dissert., Wurzburg, 1932.

13. Ponhold, H.: *München. med. Wchnschr.* **18**:692, 1938.

14. Rice, C. O.: *Am. J. Cancer* **25**:2301, 1931.

As with carcinoma, invasion of the trachea is not uncommon with hemangioendothelial sarcoma. It was present in cases 2 and 6 of Hedinger,² in 3 cases (including that reported here) in the material at Bern, and in the cases of Usui,⁶ Winnen⁷ and Wülfing.¹¹ De Quervain¹⁵ pointed out the marked tendency toward excessive and atypical bleeding of hemangioendothelioma arising in old adenoma and regards this feature under certain conditions as evidence for a presumptive diagnosis of hemangioendothelioma. The metastases, like the primary tumor, show a tendency toward excessive bleeding, which may be so extensive as to lead to death. Thus, in the case of Blum,⁹ death was due to a massive intrapleural hemorrhage from a metastasis of a malignant hemangioendothelioma of the thyroid. In the case reported here death was presumably due to massive hemoptysis from a metastatic intratracheal lesion, and in 1 other case observed in Berne but not reported, death was due to uncontrollable hemorrhage from a metastatic lesion in the mandible at the alveolar process.

REPORT OF A CASE

A 58 year old white man was admitted to the Inselspital (clinic of professor de Quervain) with the following complaints: progressive shortness of breath for over one year, pain in the region of the left shoulder for the past few months, hoarseness for the past two months, difficulty in swallowing for the last several weeks, bloody sputum for the last five days and marked loss of weight. He stated that the "goiter" which he had had for the past thirty-three years had grown considerably in the last year.

History revealed that the patient had never had pneumonia, pleuritis, fever or night sweats. He admitted that he had used external medicaments on the goiter but had never taken anything by mouth. The family history was not relative except for the fact that his brother had a "goiter."

The patient was emaciated, with marked pallor of the skin and mucous membranes and with cyanosis of the lips and hands. The pulse rate was 120 per minute; the blood pressure was 140 systolic and 90 diastolic, and the respiratory rate was 20 per minute, with mild stridor and coarse tracheal rales. The patient was afebrile. A Horner's syndrome was present on the right side and was well developed.

The thyroid gland was markedly enlarged, measuring 16 cm. in width and from 11 to 13 cm. in length. The thyroid was firm in consistency except that the lower middle part felt cystic. The surface was smooth save for occasional irregular areas. The skin was tightly stretched but was freely movable over the gland. Posteriorly the mass was firmly fixed to the organs of the neck. On the right, behind the sternocleidomastoid muscle was an irregular firm mass, measuring 5 to 6 cm., which posteriorly appeared to be continuous with the enlarged thyroid. The lymph nodes of the neck were enlarged and firm. The right superior thyroid artery was enlarged, firm and palpable. The left could not be felt. The trachea was pushed toward the right but was not palpable.

The thorax showed a mild left kyphoscoliosis, and on auscultation the lungs disclosed throughout moist coarse rales.

The rest of the findings on physical examination were essentially negative.

15. de Quervain, F.: *Deutsche med. Wchnschr.* 52:605, 1926.

Initially the blood showed a hemoglobin content of 67 per cent (Sahli), 3,336,000 erythrocytes per cubic millimeter (anisocytosis, anisochromia) and 14,120 leukocytes, with polymorphonuclear leukocytes 82 per cent, lymphocytes 11.5 per cent, monocytes 4.5 per cent and eosinophils 2 per cent. The sputum was almost pure blood. Microscopically the sputum showed innumerable red and white blood cells and a mixed bacterial flora but no tumor cells.

Two days after admission the patient had hemoptysis, producing approximately 1 liter of blood. The hemoglobin content dropped from 67 to 46 per cent, and the patient went into mild circulatory collapse. He was given supportive treatment. On the fourth hospital day, following intravenous and intra-



Fig. 1.—Gross specimen with the trachea opened posteriorly. Note the invasion directly under the spreader.

muscular injection of a coagulant and intravenous injection of 10 per cent sodium chloride, the patient had a chill. He continued to bleed by mouth and on the fifth hospital day was given a hemostatic intravenously and intramuscularly without any apparent influence on the bleeding. The hemoglobin content fell to 30 per cent, and there were signs of vasomotor collapse. On the sixth hospital day the patient was given a transfusion of 700 cc. of whole blood. He failed to show improvement and died on the next (seventh) hospital day.

Autopsy.—(Prof. W. C. Wegelin.) This examination was made approximately twenty-six hours after death. Only the pertinent observations are described.

The body was that of a well developed but poorly nourished white man aged 58 years and weighing 63 kilograms.

The esophagus, larynx and trachea were filled with bloody fluid. The mucous membranes of the esophagus and larynx were pale. The trachea was markedly compressed on the left and displaced to the right. In the region of the thyroid the mucous membrane of the trachea was replaced by a slightly elevated, irregularly shaped, flat, dark red tumor mass, 2 by 3 cm. in diameter, which was continuous with the left lobe of the thyroid. In the immediately surrounding area multiple flat nodules of dark red tumor, varying from 3 to 5 mm. in diameter, were seen in the mucous membrane of the trachea. These were more marked on the left than on the right and extended up as high as the subglottis. In one of these nodules, which measured 4 mm. in diameter and which lay, somewhat isolated, in the lower anterior part of the trachea, a darker red spot, 1 mm. in diameter, was seen.

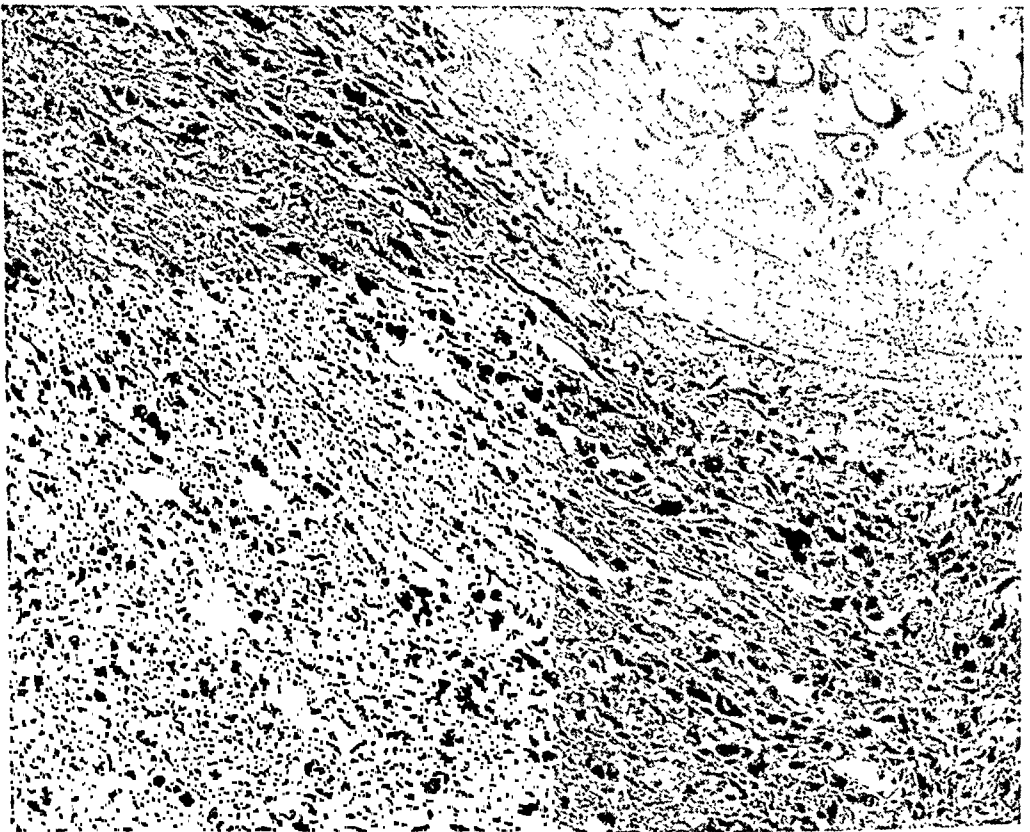


Fig. 2.—Section through the trachea. A portion of the cartilage ring is seen in the upper right hand corner. Note the slitlike spaces, some containing blood cells. Note the tendency to flattening of the tumor cells lining such spaces. Hematoxylin and eosin; $\times 150$.

The left lobe of the thyroid consisted of a firm tumor mass, measuring 11 by 7 by 7 cm. This was firmly and intimately connected to the trachea. The left carotid artery and left jugular vein were patent. No tumor thrombi were present in any of the vessels of the neck. The left lobe of the thyroid was surrounded by a thick, dense connective tissue capsule, 2 mm. in width. The cut surface of this portion of the thyroid showed a large irregular cavity, filled with partially fluid and partially inspissated bloody material. The former was dark red and the latter was chocolate brown and friable. After removal of this material the tumor tissue, which varied from red to grayish red, was seen lining the wall of this

cyst. Within the tumor tissue were innumerable, irregularly distributed slitlike spaces filled with dark red fluid. In some portions of the cyst, just peripheral to the tumor tissue and within the fibrous capsule, a dark brown and somewhat transparent layer of homogeneous material, containing a few slits, was seen. On the tracheal aspect the tumor tissue was found to be directly continuous with the larger tumor mass lying within the trachea.

Anteriorly, extending from the thyroid cartilage to the suprasternal notch, was a roughly ovoid cyst from 10 to 11 cm. in diameter. This cyst, which was attached to the anterior cervical muscles, was filled with a thick, light brown fluid containing a large amount of crumbly, friable material. The cyst was surrounded by a firm wall, from 2 to 3 mm. in thickness, in which was found dark red and brown friable material similar to that seen within the cyst.

The right lobe of the thyroid was also enlarged, measuring 9 by 3 by 3 cm. The cut surface showed many oval or round encapsulated nodules, measuring from 2 to 4 cm. in length and each about 2 cm. in width and thickness. These nodules, which varied from grayish red to brown, were slightly hemorrhagic and transparent. In the upper pole an occasional nodule, gray and measuring about 0.5 to 1 cm. in diameter, was seen between compressed lobules of thyroid tissue.

The cervical lymph nodes on both sides were markedly enlarged, firm and dark red. Many of the nodes showed on the cut surface hemorrhagic areas of softening lying in dark red to grayish red tissue from which bloody fluid could be scraped. The mediastinal lymph nodes were similar.

The other organs were not remarkable from the point of view of this report, and the only reference to them will be included in the final diagnosis.

Histologic Examination.—A section taken through the trachea showed extensive destruction of the entire tracheal wall and mucous membrane. Only the cartilaginous rings were intact. The tumor cells replacing the wall showed a rich variety of shape and arrangement. In general, the cells were roughly polyhedral, with abundant, poorly defined cytoplasmic outlines. The cytoplasm took a pale blue stain, was homogeneous and in some situations was pale bluish red. Many of the cells were phagocytic and contained fragments and whole red blood cells. Other cells were more spindle-like in form, and these were somewhat darker in color. The nuclei of the tumor cells were extremely prominent, with sharply defined nuclear membranes. The nucleoplasm was sparsely granular, and the nuclei contained one or more accentuated dense dark blue nucleoli. Nuclei of irregular size and shape were encountered, and atypical mitotic figures were infrequent. An occasional multinucleated cell was seen.

In many situations the tumor cells lay loosely in a scant fibrillar connective tissue stroma, in which many red and white blood cells were enmeshed. Here the appearance was typically sarcomatous. In other situations were encountered well defined slits, spaces and tubelike structures, lined either by single or by multiple layers of cells. These structures simulated small vessels and capillaries and were lined by robust tumor cells. Many of these spaces contained red blood cells while others were empty. Various degrees of flattening of the tumor cells lining these spaces were noted until vessels indistinguishable from normal capillaries were seen. Mucous glands and mucous membrane were completely destroyed.

Sections taken from the wall of the cystic left lobe of the thyroid showed no recognizable thyroid tissue. The inner surface of the cyst was lined by a pale red amorphous layer of "caoutchouc hyalin," containing no cellular elements but impregnated with granules of hemosiderin. In this layer numerous acicular slits were seen, and the outer portions were slightly calcified. Peripheral to this layer was a dense connective tissue capsule, in which collections of tumor cells

were present. These tumor cells were spindle shape, lay in compact masses and were supported on a fibrillar connective tissue stroma. The cytoplasm was dark and poorly defined, and the nuclei were dense and not particularly prominent. No vascular or slit formation was present here, and the appearance was that of a simple spindle cell sarcoma. In some situations massive hemorrhage was seen in the capsule. Here the tumor cells had phagocytosed hemosiderin and red blood cells. In order to preserve the specimen as nearly intact as possible, no further sections of the tumor mass were made, but Professor Wegelin was convinced that further section of this lobe would show characteristic areas, similar to those seen in the trachea and in the lymph nodes.

A section made from the nodular right lobe of the thyroid showed no tumor tissue, but here a few follicles of glandular tissue with scant colloid still remained in the peripheral portions of the adenomas. A few compressed and atrophic follicles of thyroid tissue were seen in the periphery. The centers of the adenomas were filled with masses of "caoutchouc hyalin," in which were found dilated and cavernous blood spaces, lined by flat endothelium. These cells did not appear to be tumor cells. They may, according to Wegelin,¹ have been either the site of endothelial proliferation as a response to the general tumorous proliferation or the original site of tumor formation.

Sections of mediastinal lymph nodes showed the major portion of these nodes to be replaced by a neoplastic growth composed of robust endothelial-like cells containing prominent nuclei. These cells were arranged in short parallel strands about blood-filled lumens or lay loosely in small clumps. In the latter situation the tumor cells were markedly polymorphic, with large amounts of cytoplasm and with round or oval vesicular nuclei. Here and there occasional spindle cells were encountered. A portion of the tumor tissue was hemorrhagic and was infiltrated by red blood cells and considerable numbers of polymorphonuclear leukocytes. In the sinus of one of the lymph nodes were many endothelial cells filled with phagocytosed hemosiderin.

Anatomic Diagnosis.—The conditions observed were: hemangioendothelial sarcoma of the thyroid, with metastases in the cervical, supraclavicular and mediastinal lymph nodes; perforation of the trachea by the tumor, with implantation metastases in the tracheal mucous membrane; nodular parenchymatous cystic goiter; pulmonary emphysema and edema; hypertrophy of the right ventricle; healed endocarditis of the mitral valve; mild generalized arteriosclerosis; atrophy of the spleen; chronic cholecystitis with cholelithiasis; enlargement of the prostate gland, and hypertrophy of the urinary bladder.

COMMENT

An attempt to determine the exact incidence of hemangioendothelial sarcoma of the thyroid proves difficult for three reasons: In the first place, the incidence of malignant disease of the thyroid varies proportionately with the incidence of endemic goiter and particularly with the incidence of nodular goiter, being greatest in those regions where nodular goiter is endemic. Second, the incidence of this type of tumor in the general population cannot be determined from the study of statistics of surgically removed thyroids or statistics which include the numbers of surgically removed thyroids, for, as Graham¹⁶ has indicated, the incidence of neoplasms of the thyroid gland in surgically

16. Graham, A.: Surg., Gynec. & Obst. 39:781, 1924.

removed material amounts to a "great exaggeration of the normal incidence." Third, the variegated and manifold histologic picture that the malignant hemangioendothelioma presents makes it appear likely that many tumors of this type involving the thyroid are variously classified by different investigators. It is only by a simultaneous comparison of the incidence of nodular goiters and the incidence of malignant neoplasms of the thyroid and uniform histologic classification of malignant tumors of the thyroid in the same and in a general autopsy population that the true incidence of this type of thyroid neoplasm can be determined. Unfortunately, such correlated statistics are seldom found in the literature, although Wülfing¹¹ analyzed the material in Freiburg in this fashion. The statistics given by the majority of investigators do not have all these factors in view or include surgical material in the totals. It is beyond the scope of this paper to go into a critical analysis of the incidence of hemangioendothelioma of the thyroid.

Tumors of few other glands offer such a rich variety and complexity of cellular form as the malignant tumors of the thyroid. In one and the same tumor and even in the metastases variations from what appears histologically to be normal thyroid tissue to complex pictures which in the same field simulate both carcinoma and sarcoma are seen. Thus there are not only considerable differences in classification and nomenclature but also divergent opinions as to the relative incidence of sarcoma as compared with carcinoma. Indeed, Ewing¹⁷ and Smith¹⁸ go so far as to question the likelihood of sarcoma ever arising in the thyroid, while Pemberton¹⁹ stated that true sarcoma of the thyroid is rare. In a list of publications given by Kassel,¹² in which the ratio of the incidence of sarcoma to that of carcinoma is quoted, the figures vary from 1:2 to 1:100. The relatively large numbers of instances of sarcoma of the thyroid reported by European workers stand in striking contrast with the occasional one of sarcoma of this organ mentioned by American pathologists. In spite of the fact that Wegelin¹ warned against indiscriminate diagnosis of hemangioendothelioma, he admits that hemangioendothelioma must be considered as one of the more common types of tumors affecting the thyroid. According to Wegelin, many tumors which on first glance have appeared to be sarcoma or carcinoma have on further careful investigation proved to be typical hemangioendothelioma.

On the other hand, he agrees with Ewing¹⁷ and Smith¹⁸ that frequently multiple sections taken from various parts of a tumor which in the original section can be called sarcoma will prove its epithelial origin and nature. However, he is of the opinion²⁰ that the same factors which produce a great incidence of nodular goiters and malignant tumors in general may be responsible for a greater incidence of sarcoma in his material as compared with thyroids in other parts of the world. It is difficult to conceive why the thyroid should be an exception to the general concept that both sarcoma and carcinoma may

17. Ewing, J.: *Neoplastic Diseases*, ed. 2, Philadelphia, W. B. Saunders Company, 1922, p. 908.

18. Smith, L. W.: *Arch. Path.* **10**:524, 1930.

19. Pemberton, J. de J.: *Ann. Surg.* **87**:369, 1928.

20. Wegelin, C.: Personal communication to the author.

arise in the same organ. The remarkable vascular alteration that attends the growth and development of adenoma frequently is marked by endothelial proliferation of varying degrees, which may well be the starting point of hemangioendothelioma.

There are many features which Hedinger² and Wegelin¹ consider macroscopically characteristic and perhaps unique for hemangioendothelial sarcoma of the thyroid. According to them, the tumor is usually solitary and more or less round or oval. It is cystic and filled with fluid or clotted blood. The blood mass is surrounded by a grayish brown transparent layer of so-called "gutta percha hyalin," containing many vascular slits filled with fluid blood. This layer is continuous with the grayish red to pale gray tumor tissue, which often contains small areas of hemorrhage or small yellow foci of necrosis. The layer of hyalin may surround the entire blood mass, but in some cases it may be present in only one portion of the periphery. The outermost portion of the tumor is usually surrounded by a connective tissue capsule, but the tumor frequently breaks through the capsule and invades the adjacent structures.

Microscopically hemangioendothelial sarcoma shows a rich variety of histologic structures. The appearance may resemble that of simple telangiectasia with widening and multiplication of well defined vessels of capillary size. In such cases the endothelial cells are irregular in size and shape and project into the lumens. As further proliferation of the endothelium occurs, the cells become larger, and their nuclei become more rich in chromatin and show mitotic figures. The phagocytic activity of these endothelial cells is marked, especially for red blood cells. In rapidly proliferating areas the endothelial cells become more polymorphic and may lie loosely intermixed with red and white blood cells. In such areas various stages of lumen formation may be seen. When tumor cells proliferate within such lumens and become large and polyhedral, the picture may closely resemble that of carcinoma. Such masses may show secondary lumen formation and become filled with blood elements, and the endothelium then becomes flatter until it resembles normal vascular endothelium. At times the microscopic picture resembles that of sarcoma, with closely packed polymorphic or spindle-shaped cells that may show only a few areas of sprouting or budding and only suggestive evidence of canalization. Occasionally, the microscopic picture may be that of cavernous spaces supported on a rich and sometimes hyalinized connective tissue stroma. These sinuses are lined by robust endothelial cells which occasionally project, like papillae, into the lumen. Limacher³ described such a case.

Wegelin¹ expressed the opinion that in the majority of cases hemangioendothelioma arises in adenoma rather than from the endothelium of blood vessels in the supporting stroma of an otherwise normal thyroid. In many instances of adenoma showing a layer of "caoutchouc hyalin," widely dilated and thin-walled blood vessels are frequently encountered, in which the endothelium is swollen and occasionally multilayered, and it is in situations such as this that he believes hemangioendothelioma may arise.

Secondary changes in hemangioendothelial sarcoma are frequent. These degenerative changes consist largely of hemorrhage and necrosis.

There is a marked tendency of tumors of this type to destroy the walls and invade the lumens of vessels. Thus, tumor thrombi are mentioned by Limacher³ and Wegelin.¹ The possibility cannot be excluded that the thrombi are formed from proliferating endothelium of preexisting capillaries which communicate with veins.

The most frequent site of metastasis of hemangioendothelial sarcoma is the lung. Metastases have been described in other situations but are uncommon. Thus, Usui⁶ described metastases in the calvarium, tibia and femur; Winnen,⁷ in the diaphragmatic pleura; Clivio,⁵ in the stomach, liver, sternum and humerus; Matti⁸ and Ponhold,¹³ in the gums and tongue, and Wegelin,¹ in addition, in the adrenals, heart, mandible, brain and choroid plexus. In about half the cases metastases are found in lymph nodes.

The majority of cases of hemangioendothelioma, according to the material at Bern, occurred in persons between the ages of 45 and 70 years, although the tumor may occur as early as the twentieth year of life. This is also true of carcinoma.

The terminology of these tumors is not always uniform. Wegelin¹ suggested that the term "hemangioendothelioma" should be employed and when used alone should indicate a benign tumor derived from an endothelial proliferation of blood vessels which has a tendency to form vascular channels. He believes that the sarcoma-like forms should be called hemangioendothelioma sarcomatoides only when the appearance suggests strongly that seen in sarcoma; otherwise, he thinks that the term "malignant hemangioendothelioma" characterizes these tumors sufficiently to include those that may in some areas resemble carcinoma. In order to conform with the more usual American terminology, this case has been designated as one of hemangioendothelial sarcoma.

SUMMARY

A case of hemangioendothelial sarcoma of the thyroid, occurring in a 58 year old white man, is reported. The tumor invaded the trachea and metastasized to cervical and mediastinal lymph nodes. The statistical analysis of the relative incidence of this type of tumor is unsatisfactory. Nevertheless, the tumor appears to be more frequent in Europe, particularly in the goitrous regions of Switzerland, than in North America. It probably arises from the vascular structure of an adenoma rather than from that of an otherwise normal thyroid.

Laboratory Methods and Technical Notes

AN INEXPENSIVE EMBEDDING OVEN FOR USE IN ROUTINE WORK ON TISSUES

S. MILES BOUTON JR., M.D., INGLESIDE, NEB.

Wherever a considerable amount of work on tissues must be done as a part of the general laboratory routine, the recurrent problem of efficient handling and economy of operation presents itself. The average small laboratory does not, in most cases, require complete automatizing and thus usually specializing of the procedure of tissue embedding. More important by far is the general usefulness of every item of equipment placed in the usually limited space at the disposal of the technician.

With these conditions in mind, I attempted a few years ago to design a combination embedding oven and emergency incubator that could be constructed of the simplest and most available materials. Although this laboratory maintains a high standard in the preparation of histologic material, the resulting equipment has given such satisfaction in several years of service that it is thought advisable to describe it here.

DESCRIPTION OF OVEN

The oven is illustrated in figure 1. It consists primarily of a box constructed throughout of $1\frac{1}{8}$ inch (2.8 cm.) lumber, thus utilizing the insulating qualities of wood. The door occupies the entire frontage, is completely recessed into the frame of the box and consists of a wood frame holding two panes of glass, separated from each other by an intervening air space. The door is supplied with an inexpensive catch lock. The inside of the box is equipped, a short distance from the bottom, with four regulation screw sockets, two in each side wall, to hold four electric light bulbs (in this laboratory, two 25 watt and two 50 watt bulbs), and the outside, with individual switches, one for each bulb. The electric cord leaves the box at the rear. The top of the box is perforated by six $\frac{1}{2}$ inch (1.3 cm.) holes (the number and diameter of these holes may, of course, vary) within a rectangular space large enough to accommodate a dehydrating jar. This space is marked off by a low wooden frame to hold the jar, and the frame, open at the rear, furnishes guides for a sliding panel which can be adjusted to cover from one to all six holes. For this purpose, the latter are staggered as indicated in figure 2.

The inside of the box is further equipped with rails along both sides, not too close to the bulbs, to hold two removable stout wire mesh trays. A centigrade thermometer is hung on the inside of the door, its mercury bulb insulated against the glass by a piece of very thick cardboard. Thus the temperature within the oven can be read at a glance without opening the door. For purposes of comparison and checking, another thermometer may be attached to the rear wall in such a manner that it also may be read through the closed door.

From the Hastings State Hospital; Juul C. Nielsen, M.D., superintendent.

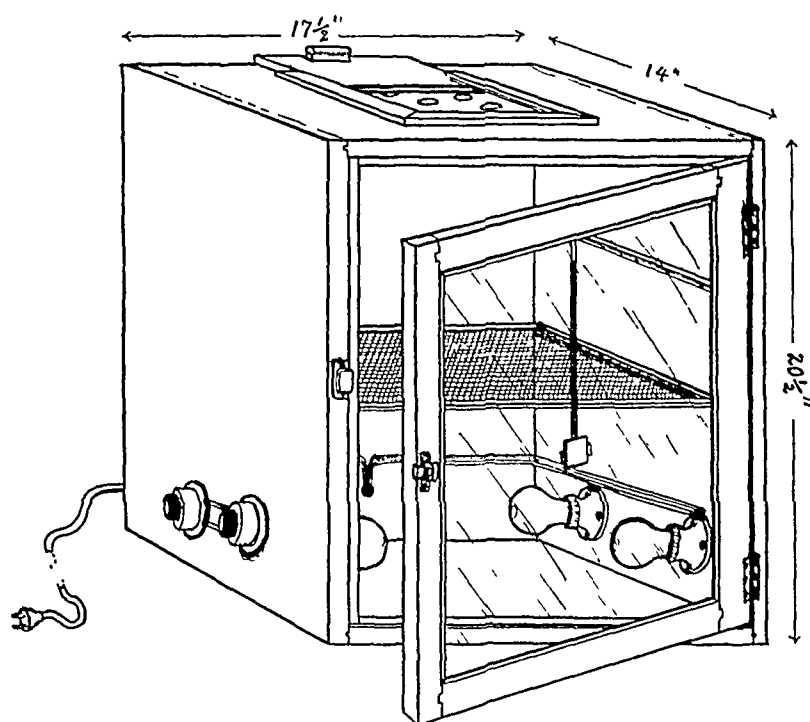


Fig. 1.—Drawing of the embedding oven.

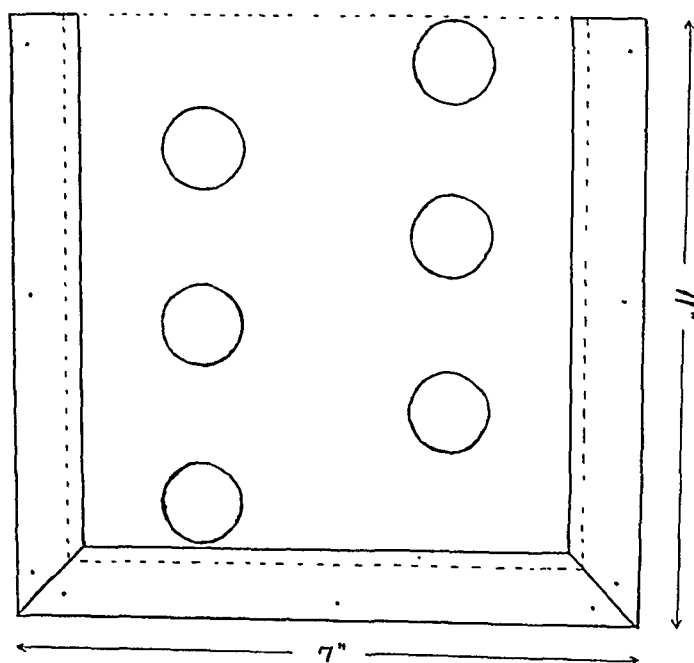


Fig. 2.—Detail: the top of the box, perforated with six holes, staggered to permit covering of from one to all six holes.

Paraffin is kept in metal cups with handles, numbered on the outside, and a mixture of xylene and paraffin (or of dioxane and paraffin) is kept in a glass jar on top of the box for prewarming and saturating the specimens before embedding.

If the box is to be used as an incubator or for any purpose which requires that specimens be kept away from light, the test tubes or other objects may be wrapped in black paper such as that in which x-ray films are shipped. In order to insure a more even temperature on all parts of the specimen, the latter may be placed in a container filled with water.

A wide range of temperature can be achieved with this equipment. With the bulbs now in use, the lowest temperature which I have been able to maintain was a temperature of about 27 C., and the highest, a temperature of about 86 C., the latter without apparent damage to the bulbs or other parts. Once the highest desired temperature has been reached (the rise is gradual), the oven will maintain this temperature on a surprisingly even level, provided the outside temperature (room) does not fluctuate too markedly while the box is in use.

All outside surfaces should be well painted, preferably in white. The approximate dimensions are given in the accompanying illustrations, and are based on the greatest degree of practicability (i. e., ease of getting at all parts of the box, visibility, available space and uniformity of temperature) but may be varied at will. This applies also to the number of light bulbs and trays. The oven described here is the third I have put in use, all three differing somewhat from one another, and is, so far, the most efficient.

The cost of construction, especially in an institution maintaining its own carpenter shop and tinsmith, is obviously extremely low, and the cost of upkeep is negligible, as the bulbs will last a surprisingly long time and use little current.

SUMMARY

A "home-made" embedding oven is described in detail, with its uses.

The chief advantages are the low initial cost, the low cost of upkeep, the great convenience in use and the wide range of usefulness.

The chief disadvantage is the difficulty of maintaining an inside temperature at a constant level if the outside temperature fluctuates too widely.

General Reviews

ELASTIC TISSUE

GEORGE M. HASS, M.D.*

BOSTON

(Concluded from page 365)

PATHOLOGY

The pathologic histology of the elastic fiber may be reviewed briefly in preparation for a general discussion of the alterations which occur in elastic tissue in abnormal bodily states. Atrophy of the elastic fiber is characterized in the early stages by partial or complete loss of specific staining qualities. This refractory stage in case a large fiber is concerned is often preceded by segmentation of the fiber. Subsequently, there is diminution in thickness and ultimate disappearance of the fiber. The assumption of basophilic staining qualities with concomitant loss of affinity for the orcein stain is another common observation. Other evidences of degeneration are skein formation of networks and aggregation of fibers into masses. The individual fibrils may show various thickenings, transverse fractures, granular disintegration, longitudinal fission, vacuolation or fatty degeneration.

In order that there may be some correlation of the pathologic data, the various abnormal states will be grouped under several headings, under which will be treated certain inflammatory, degenerative, neoplastic, vascular, endocrine and congenital diseases, as well as mechanical or chemical factors.

Inflammatory Diseases.—Inflammation is almost always accompanied by deterioration of the elastic tissue. The degree and the rapidity of degeneration vary in different inflammatory processes. A satisfactory explanation for this variability has not been given. Certain general rules, which apply principally to the type of the inflammatory response rather than to its etiologic nature, have been advanced. There are reasons, however, for believing that different etiologic agents may exert different influences. The chief deleterious factors have been attributed to inundation of tissues with alkaline fluids, accumulation of hypothetic toxins, infiltration by inflammatory cells, proliferation of fibroblasts and especially deposition of collagen.

The acute exudative processes usually are characterized by early disappearance of the staining capacity of the elastica, with thinning of the fibrils, followed by almost complete disappearance of networks at

* Past Member of the Society of Fellows, Harvard University.

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the height of the process (Unna, 1896). In simple erythema there is no appreciable disturbance of the elastica (MacLeod).

Mechanical crushing does not seriously injure the fibers, but they succumb quickly in the area of inflammatory response (Katsurada). Within six to twenty-four hours after crushing a tissue, Katsurada found slight diminution in the amount of elastica and in the staining capacity of the fibrils. Five days later almost all fibers had disappeared.

The effect of heat has been studied (Unna, 1896). Dry heat caused disappearance of elastic networks as such, but those places where they normally were present elected the specific stains. The regional collagen was either covered by, or had a tendency to imbibe, the residual elastin. Boiling water produced similar changes in the elastica, but no elastin was imbibed by the collagen. Luithlen did not agree with Unna. He contended that burning was not injurious to the elastica.

In bronchopneumonia Sawada failed to find any definite atrophy of elastica. Jores (1902) showed that the apparent diminution of elastic tissue in lobular pneumonia was at least partly dependent on the loss of the normal staining reaction. In chronic pulmonary consolidation Ponfick found the elastica either reduced to a few scattered remnants or invisible.

In erysipelas, phlegmonous infiltration of the cutis and purulent processes the elastic tissue undergoes extensive degeneration. In erysipelas it perishes from colliquation, the early stages being characterized by loss of specific staining qualities and thinning of fibrils, while at the height of the reaction the networks are rarely visible (Unna, 1896). Similar changes have been found in phlegmonous infiltration of the cutis. In these instances segmentation, loss of staining reaction and thinning of fibrils represent successive stages of degeneration (Jores, 1902). In purulent processes there are, in addition to purulent disintegration, segmental degeneration of large fibers and atrophy of delicate fibrils. The elastica may remain in this state of degeneration for a long time (Jores, 1902). B. Fischer described similar changes in the walls of veins which were involved by purulent thrombophlebitis.

Various changes in a few other lesions of exudative nature have been described. The elastica largely disappeared below lesions of dermatitis herpetiformis (White) and pemphigus (Luithlen). Luithlen stated that hemorrhagic lesions and the cutaneous lesions in variola did not affect the elastica. Kreibich could not fully confirm these observations. In contrast to the general rules in inflammation, White found an increase in the elastica of the corium and vessels beneath an ulcer caused by the diphtheria bacillus.

Productive inflammatory processes, especially those of a chronic nature, are usually characterized by the most extreme degree of degeneration of elastic tissue. It is in these lesions that cellular infiltration, fibroblastic proliferation, the formation of granulation tissue and cicatri-

zation exert those effects by which a more or less permanent and complete degeneration or atrophy of the elastica is brought about.

Tuberculosis serves as a classic example of this type of lesion. In the granulation tissue of tuberculosis the elastica degenerates rapidly and extensively (du Mesnil de Rochemont; Krösing and Passarge; Unna, 1896; Guttentag; Lewinsberg, and others). Schmaus found that the primary tubercle is not always depleted of elastica but that the greatest injury occurs in the vascularized granulation tissue. Also there may be a regional zone of atrophy around tuberculous areas, even though the remaining structural elements are normal (Jores, 1902; du Mesnil de Rochemont). In lupus erythematosus, which is questionably of tuberculous origin, Unna (1896) found the elastica unusually normal even where collagen had disintegrated. Melnikow-Raswedenkow described degeneration of the elastica in tuberculosis of the testis; Orth confirmed his findings. Federmann believed that in the early stages of testicular intracanalicular tuberculosis the elastica disappeared first in the walls of the tubules which were involved by the granulation tissue. In the caseous areas Melnikow-Raswedenkow noted that the elastica was not seriously injured. This is in accord with his observations on the good state of preservation of elastica in the necrotic portions of splenic infarcts and with those of Passarge in nonsuppurative gangrene of the skin. Federmann concluded that the more rapid and widespread the caseation necrosis the less was the destruction of elastica. When softening occurred in the caseous areas, the elastica was destroyed rapidly.

The injury to the elastica in tuberculosis is so prominent that this feature has been used in the differential diagnosis between syphilis and tuberculosis. This difference stimulated several authors to postulate a theory of specific elastotoxins. Wechsberg, an advocate of this theory, found that the lesions produced experimentally by emboli of tubercle bacilli in the lungs were characterized after six hours by atrophy of the elastica of the involved vessels. After forty-eight hours there was degeneration of the elastica between the alveoli.

The fate of the degenerated elastic fibrils has been commented on by many authors. Unquestionably, many are destroyed completely. Unna (1896) contended that no new elastic fibers were formed in the cicatrices of tuberculosis but that during cicatrization remnants of elastica might be included in the scar tissue. The possible role of the giant cells is of interest. Ssudakewitsch and Unna (1896) demonstrated remnants of fibers in these cells. Some fibrils stained well and others poorly. Lewinsberg found that there was a direct proportion between the number of giant cells which contained fibrils and the amount of elastica in the tissue. Rona was able to show that calcific clumps in giant cells were calcified fragments of elastica. He assumed that the calcification occurred within giant cells because the extracellular fibers contained no calcium. He

demonstrated also that there was deposition of iron as well as of calcium around the phagocytosed fibrils.

Federmann studied syphilis of the testis. In early interstitial orchitis the elastica in the walls of seminiferous tubules remained intact. So soon as the inflammatory process involved the walls and lumens of tubules the elastic fibers became separated, and eventually they disappeared. The interstitial tissue in fibrous interstitial orchitis contained a few elastic fibrils, which may have been remnants of injured elastica or possibly new-formed fibrils. In contrast to tuberculosis, the fibers around the tubules were retained, even though the lumens of tubules were often completely obliterated. Gummas were similar to the caseous lesions of tuberculosis in that there was preservation of elastic networks until softening or purulent necrosis occurred. At this stage the elastica promptly degenerated.

Krösing described atrophy of elastica in the zones of exudation and cellular infiltration in syphilitic papules. After the exudate had been resorbed, he found that the elastic networks presented an essentially normal appearance. From these observations he concluded that the fibers which had lost their staining qualities during the active phase of the inflammation had regained their affinity for elective stains after the subsidence of the reaction.

Unna (1896) in a study of the primary lesion of syphilis found that at the height of the development of the chancre all elastica had disappeared.

Other diseases which are characterized by the proliferative nature of the inflammatory reaction show rather extensive degeneration of the elastica. By the time that the leprous nodule is well formed, the elastic fibers terminate abruptly at the periphery of the nodule and are absent in the interior. Unna (1896) stated that no new fibrils are formed. Fragments of elastica encrusted with iron and calcium were found by Rona in the giant cells. Fibers also were found in the giant cells in pascha-churda (Ssudakewitsch) and cutaneous sarcoid (Hektoen), both being diseases in which the elastica is destroyed. In the granulomatous areas of frambesia (White) and cutaneous blastomycosis (Unna, 1896) the elastica is practically absent.

The less productive inflammatory processes are characterized by deterioration of the elastica, which usually is not as complete or as widespread as in the so-called typical granulomatous lesions. In ulery-thema sycosiforme (Sack) and in *ulcus serpiginosum* (Unna, 1896) there is atrophy of elastica in the areas of cellular infiltration. Unna (1896) stated that in eczema the elastica is destroyed only in areas of massive cellular infiltration. Du Mesnil de Rochemont found that in chronic eczema the atrophy was in the neighborhood of the areas of great

cellular infiltration or perivascularitis. In dermatitis exfoliativa the elastica is normal except in the areas of cellular reaction (White). White found that in pityriasis rosea there is great reduction in the amount of elastica in the papillary and subpapillary layers of the cutis.

Other conditions which are characterized usually by a slight degree of inflammation may affect the elastica. White found that in Paget's disease of the skin there is atrophy in areas of cellular reaction but hyperplasia adjacent to these areas. In molluscum contagiosum he found diminution or complete absence of elastic fibrils. Jadassohn stated that in striae gravidarum the atrophy is preceded and accompanied by chronic inflammatory cell infiltration. In these areas of infiltration the degeneration of elastica is similar to that which is found in atrophia maculosa cutis. Heuss confirmed these observations and contended that atrophy of elastic tissue may proceed without concomitant degeneration of collagen. Huber described diminution of elastica in the areas of cellular infiltration in atrophia idiopathica diffusa progressiva. There may be thickening of elastic fibers in scleroderma, although partial destruction of the elastica is common (MacLeod). In an instance of bronchial asthma Wawerla found degeneration, calcification and iron impregnation of elastic fibers in the vessels, bronchi and stroma of the lungs. There was a foreign body reaction around degenerated remnants of elastic tissue. Pagel in a study of several cases of bronchial asthma concluded that the only significant alteration in the elastica is an increase which may occur in the walls of medium-sized bronchi.

Cicatrization has been studied by several observers. Unna (1896) believed that granulation tissue becomes free from elastica before it is converted into scar tissue. He contended that elacin and rare elastin fibers may be formed in cutaneous scars. These fibers never attain the systematic architectural arrangement of normal networks. Jores (1900) stated that no elastic tissue is formed in granulation tissue. He found by experiment that many days or months elapsed before elastica had regenerated in appreciable amount in cicatrices. In those scars where there was great sclerosis or interstitial inflammatory reaction the elastica was always absent or sparsely distributed. He was not able to find elastic tissue in keloids. In regard to connective tissue overgrowth, it seems peculiar that in cirrhosis of the liver and in renal cicatrices elastic fibers often have been found where none was present previous to the injury.

It is quite probable that as a result of various lesions the state of preservation or the degree of regeneration of elastica may govern to some extent the nature of the scar. MacLeod stated that if elastic fibers were destroyed in an infectious granuloma, an atrophic scar was the result. He contended that if elastic tissue forms an exoskeleton which supports the collagen, a simple rupture of elastic networks will allow the

collagen to expand, the superficial furrows of the skin will disappear and a smooth scar such as is found in striae and syphilitic rhagades will be the result. It is only fair to add here that much work remains to be done before one may gain an understanding of the peculiarities of cicatrization, not only in regard to certain disease processes but also in relation to the variation among different persons.

Vascular Disease.—Whenever mention is made of pathologic changes of the vascular system, the mind is inclined to dwell on those inevitable changes which accompany and often blight the advancing years. These vascular changes have been divided into two groups, one representing the decay of age and the other the enigma of what some choose to call atherosclerosis. Many consider them as the result of distinct and separate processes. Each may well gain its end with seeming indifference to the other. More often they unite to cut short the span of life.

The effects of age become manifest soon after life has begun. For the most part they pursue a relentless course, retarded in some persons and hastened in others, but still adhering to an established sequence. Early in fetal life the aorta and other arteries show progressive increase in the connective tissue framework. This is accompanied by gradual reduction in the elasticity of the vessel. This process varies in the different arteries as well as in the tunics of the same artery. It may be studied readily in the intima, where there is gradual increase in the amount of elastic tissue as well as in that of collagen. The increase in age favors the eventual predominance of the latter. During the period from birth to middle life, the original internal elastica lamina in certain larger arteries and in the aorta splits into two or three layers. This increase in elastica soon ceases, and in the senile period the new formed laminae may atrophy and disappear while the collagen is retained. Also, age brings increase in the elastic and collagenous tissue of the media. Similar alterations in the tissue elements of the adventitia are of less significance. Thus, certain changes occur which dispose the vessel walls to become thick and firm. The concomitant loss of elastic tone allows for dilatation, elongation and tortuosity of vessels. These changes are well recognized and may be detected as readily in the retinal arteries as in the aorta. A similar process may progress with remarkable rapidity in such vessels as those of the placenta, where the span of life of arteries is but a few months.

The means by which these changes are brought about have been considered by Wells. He believed that the aging of vital tissues is analogous to the aging of colloidal gels. In both processes there is decrease in hydration with advancing age. This is associated with decrease in elasticity and flexibility. Inasmuch as repeated alterations

in degrees of dispersion of colloidal gels or emulsions diminish their capacity for returning to the original degree of dispersion, Wells expressed the interesting idea that this rule may be applied to the vital elastic colloid, elastin. In support of this is an extreme example: Apparently elastic fibers which have been damaged by overstretching are particularly prone to undergo calcification and iron incrustation.

One of the most common morphologic changes in the elastica of aging vessels is "granular" degeneration. Wells considered this as possibly a late visible stage in the dehydration of elastin, being evidence of aggregation of colloidal micellae. At first this is more prominent in the inner part of the media. Gradually it progresses toward the adventitia. When the degeneration is great, the granules collect in small clumps between the lamellae. The large lamellae of elastica are unequally swollen and show areas that stain poorly with orcein. These parts atrophy so that the concentric bands appear to be discontinuous. Some fibers show the elacin reaction. Schultz was able to follow the progressive change of elastin into elacin, the process beginning in childhood and increasing with age. Granular degeneration and diminished affinity for elective stains are found commonly in the inner sheaths of the internal elastic laminae of small and medium-sized arteries. Ribbert, Reich and Jores (1902) found a definite relation between granular degeneration of elastica and fatty deposits in the intima.

There is a tendency for aging or overstretched elastic fibers to imbibe or absorb substances of low solubility. Because of this property, the fibers become impregnated or coated with such materials as calcium, iron and silica. There is no apparent uniform proportion between the amounts of each which are deposited. The microscopic structure of the calcification often shows a radiating or concentric arrangement, which supports the theory that the deposits are formed under the rules which govern the deposition of crystalloids in colloidal matrices under the influence of colloidal forces (Rusznayk).

It has been said that normal arteries contain a small quantity of calcareous material (Ribbert; Klotz; Faber). This can be demonstrated in the first years of life and occasionally before birth. Later in life it occurs chiefly in the elastic lamellae of the media of elastic arteries. Also, calcification of the internal elastic membrane is common. Here calcium granules may be found either in or alongside the fibrils, which are often undulate or fragmented. Ameseder found that in the human aorta decrease in elastin is proportional to increase in inorganic substances.

Ravault (1929) studied calcification of arteries by the method of microincineration. He found calcific deposits in the media of aortas that were histologically normal. He believed that the calcium was

associated with elastic elements and occasionally was incorporated in the elastic substance. Furthermore, he stated that the media adjacent to the calcified intima of diseased vessels is often uncalcified and shows less than the expected normal content of ash. In the Mönckeberg type of medial calcinosis he found the mineral deposits in and around the collagenous and elastic elements rather than primarily in muscle fibers.

Atherosclerosis, according to most authorities today, is a process that is fundamentally distinct from the process which is discussed in the preceding pages (Aschoff). Although degeneration of the elastica often may precede or accompany the deposition of atheromatous substances, the two processes fail to exhibit any impressive parallelism. In fact, Anitschkow stated that in experimental cholesterol atherosclerosis of rabbits the elastic lamellae act as barriers to the infiltrating lipoids and that there is no fatty infiltration of the elastic laminae such as may be found in man.

Other pathologic states which may involve the elastica are accumulations of so-called "mucin," "hyalin" or "amyloid" in the vascular wall. The extent to which degeneration of elastica is dependent on these substances is unknown. There is no incontrovertible proof that one process is intimately allied with the other. Schultz demonstrated "mucin" in arterial walls. He believed that it had an affinity for fat and calcium. The deposition of hyalin and amyloid may be so extensive that elastica cannot be demonstrated. In these instances it has degenerated completely, has lost its elective staining qualities or has become obscured.

The effects of mechanical injury to the elastica of vessels and the result of such injury have received careful study. Jores (1900) described a new formation of elastica in the intima of vessels within eight to ten days after ligation. This was well developed within twenty days. Later (1902) he was able to show that after traumatic injury to the vessel wall abundant regeneration of elastica occurs. In this regard it may be said that new-formed vessels eventually may gain a full complement of elastic networks and that formation of elastic tissue in organizing thrombi is of common occurrence. Petroff and Glasunow found that lesions caused by pinching or cauterization of arteries lead to increased permeability of the arterial wall. The subsequent changes in arteries of the elastic type depend on the degree to which the elastic framework of the vascular wall has been injured (Ssolowjew 1929). Great injury results in replacement by granulation tissue, which arises at first in the adventitia. If all elements except the elastica are injured, there is regenerative proliferation of the cellular parts of the uninjured media. In this repair new elastic fibers may be formed.

The role of the elastic networks in the formation of aneurysms was discussed at great length by early authors. Von Recklinghausen, Helm-

städter, Manchot and Eppinger believed that the basic factor leading to the development of the aneurysm is a rupture of elastic fibers. They contended that the sharp interruption of the continuity of the elastic lamellae favored the thesis that the rupture is of mechanical rather than of inflammatory origin. Koester, Krafft and Zmudeeg considered these ruptures as secondary to inflammatory processes.

Thoma emphasized the significance of altered elastica in the pathogenesis of arteriosclerosis. He directed attention to small fissures or defects in the media. These seemed to be independent of the state of preservation of the regional elastica. He believed that in addition to the disruption of the elastica there is an involvement of smooth muscle cells and connective tissue. He commented on the presence of cicatrices and leukocytes in the region of tears and noted that degeneration of the medial elastica usually is found only where there is a fairly cellular overgrowth of connective tissue. Ssolowjew (1930, 1932) produced isolated tears in the elastic lamellae of the inner layers of the media by mere displacement of the carotid artery. The tears appeared after a lapse of time. The process of repair eventually was completed by replacement of the tears chiefly by intimal plaques that contained elastic elements.

If one assumes that the rules for elastic colloids *in vitro* are applicable to elastic tissue, repetition of extension, even within physiologic limits, soon will reduce the elasticity of this tissue and increase its elastic resistance. Apparently elastic fibers are damaged by overstretching, and it is said that fibers so treated are prone to bind calcium and iron. This may be of interest in relation to hypertension. It has been known for a long time that chronic hypertension and reduplication of elastic laminae in the intima of renal arteries usually occur together. A similar, although somewhat different, picture is found in the compensatory adjustment of an artery to a decreased blood flow. In this instance, loose collagen and elastica symmetrically thicken the intima and thereby reduce the size of the lumen. The several types of arteritis of specific or nonspecific nature show various degrees of deterioration and reparative regeneration of the elastica. These usually follow the rules which govern the general changes in inflammatory conditions. They have been considered elsewhere and need no repetition.

Thromboangiitis obliterans, because of its peculiar character, may be considered in more detail. Similar pathologic changes may occur in Raynaud's disease, arteriosclerosis and scleroderma. Krompecher (1930) believed that the stenosis of the lumens of certain vessels was produced primarily by proliferation of specific cells, "the elastoblasts." He demonstrated a delicate elastic membrane around each elastoblast. He con-

tended (1931 [c]) that formation of elastica occurs only if these cells are present and that these cells degenerate as soon as the elastic elements are fully formed. The same process may be repeated in such a way as to cause stenosis of the lumens of the vessels which form in thrombi. Jäger made a careful study of thromboangiitis obliterans. He found that during the acute stages the elastica of the vessels was chiefly unstainable. In later stages the elastica often showed variable changes. The external elastic membrane frequently was disrupted and calcified. There was rarely a foreign body reaction to the remnants of elastic fibers. The adventitia usually showed an increase in the elastica. The chief findings in small vessels were folding of the internal elastic membrane, intimal thickening and in the later stages increase in the elastic fibers of the intima.

Although not of inflammatory origin, the alterations which occur in the ovarian and uterine arteries under various physiologic stimuli are of unusual interest. Woltke, Kon and Karaki and Böshagen described medial degeneration and endarterial formation of new vessels in the arteries around ovarian follicles. Eden and Lockyer described a similar process in uterine arteries. The peculiar changes which have been designated as ovulation and menstruation sclerosis were studied carefully by Sohma. Before the establishment of menstruation the cortical arteries of the ovaries are devoid of elastic tissue except for a single internal elastic membrane. With the beginning of the menstrual periods an elastic network appears in the muscularis and often condenses to form an elastica externa. During menstruation there is distention of arteries. Serous exudate permeates the walls, and fibers rupture. After menstruation a network of elastic fibers reforms in the media. Similar changes occur in vessels around a ripening follicle. After rupture of the follicles there is great increase in the medial elastica, and the muscle is replaced by a fibroelastoid substance. Beneath the vascular endothelium a new media appears. This displaces the degenerated media toward the periphery. This process may be repeated during several ovulations so that the elastic remnants of two or three medial layers which have degenerated in succession may be found encircling the vessel.

Similar degeneration and reformation occur in uterine arteries on termination of pregnancy. At the menopause the arteries of the uterus and ovaries show atrophy of the peripheral medial remnants and of the internal well formed media. During this involutional period senile arteriosclerotic changes commonly are superimposed on the physiologic retrogression.

The rapid involutional changes which occur in the placental arteries and the ductus arteriosus are somewhat analogous but not identical to those which are described in the foregoing paragraph.

Little seems to be known about lesions of the elastica which may be produced by toxins or those which may occur in "allergic" responses. The deleterious effects of diphtheria toxin on the walls of blood vessels are well known. Many authors have contended that epinephrine is injurious to the vessels. The sclerosis induced in rabbits by experimental administration of epinephrine occasionally is characterized by stretching and disintegration of the elastica. These changes are late manifestations and follow the deposition of calcium granules in the ground substance. There may be new formation of intimal elastica. These are not specific changes because many substances produce alterations similar to those observed in epinephrine-induced sclerosis (Anitschkow).

The elastica of veins may be affected in many ways. The changes occurring with advance in age are similar to those which occur in arteries, but there is less hyperplasia of the elastica. Increase in collagen commonly occurs throughout the wall, but atheromatous and calcific deposits are rare. The most important changes are those which result from stasis of blood. In such instances there is primarily degeneration of smooth muscle cells and of elastic fibers in the media. New-formed elastic and collagenous fibrils commonly appear in the intima adjacent to the areas of degeneration and fibrosis in the media (Scagliosi). In acute phlebitis and purulent thrombophlebitis widespread degeneration and disappearance of elastica often occur (Fischer).

Degenerative Diseases.—This is not a satisfactory subgroup, but it includes certain conditions which are characterized by atrophy and degeneration of the elastica, independent for the most part of mechanical or inflammatory influences. Among these conditions certain cutaneous diseases, especially those which accompany advanced age and certain degenerations of blood vessels, have received the most study.

Senile atrophy of the skin is accompanied by important alterations in the elastic networks (Schmidt). In the skin of the face the fibrils become swollen. They undergo granular degeneration and gather in hyaline masses. The swelling of the fibrils is either diffuse or in the form of numerous varicose thickenings so spaced as to suggest a rosary. The fibrils may either retain their specific staining reaction or develop an affinity for dyes that are rejected by normal fibers. In the second form of degeneration the fibrils disintegrate into granules which are highly refractile and which retain the elective staining qualities. This type of deterioration finally may lead to formation of strands or irregular clumps of granules, which tend to coalesce so as form homogeneous masses. Schmidt believed that in this way hyaline masses which have an affinity for the elastic stains are formed. These may become so numerous that in certain severe cases structureless homogenous masses replace a large

part of the upper cutis and in rare instances may be distributed throughout the entire cutis. Reizenstein, Krösing and Passarge and Krzysztalowicz, however, contended that the strands and clumps are comprised of a maze of very delicate fibrils rather than of conglomerate masses of fused granules.

Unna (1896) found that some of the fibers in senile skin which stain weakly with orcein have an affinity for basic aniline dyes. He believed that there was some chemical alteration of the substance of the fibrils. He named the changed substance elacin to distinguish it from elastin of normal tissue elements. The substance elacin is found especially in senile skin and in other conditions in which atrophy is prominent. In addition to alterations in the elastica, he described changes in the collagen of senile skin. The substance of fiber structures which have the physical appearance of collagen but the staining qualities of elastin, he named *Kollastin*. The substance of collagenous fibrils which have the staining reaction of elacin was designated by him as *Kollacin*. In colloid degeneration of the skin he believed that the "colloid" is formed from *Kollastin*, and that with its appearance the specific elastin stain is lost.

Reizenstein found that the changes described in the elastica of senile skin are not restricted to the skin of old people but are detectable in persons as young as 26 to 28 years of age. Unna (1896) confirmed this and concluded that the "senile" type of degeneration of elastica is less dependent on age than on climatic influences. Krzysztalowicz agreed with Unna. He described elacin in the upper layers of the cutis in young persons and in the deeper layers in older persons. *Kollastin* appeared in the upper layers of the skin of young persons, while *Kollacin* formed in larger amounts in aged persons. He noted that the various changes were more prominent in men than in women, especially among the laboring classes. Furthermore, the exposed parts of the body occasionally were the only areas which showed changes.

Himmel emphasized the occurrence of areas of cellular infiltration in the skin. He believed that the "senile" changes in the elastica might be attributed to inflammatory reactions to unknown deleterious agents.

There are a few other cutaneous lesions in which the elastic tissue shows changes that may be considered degenerative. In striae gravidarum and striae distensae the beginning of the deterioration of the elastica is associated with chronic infiltration of the tissues by inflammatory cells. This fact does not exclude entirely the possible importance of hereditary, mechanical or endocrine factors. These will be discussed elsewhere. Jadassohn found that the degeneration of elastic tissue in atrophica maculosa cutis is similar to that which occurs in striae gravidarum. Huber described significant diminution of elastica in atrophica idiopathica diffusa progressiva. This occurs chiefly in areas

of cellular infiltration in the subpapillary zone of the cutis. The atrophy of elastica which may result from prolonged edema and that which may occur in scleroderma will be considered elsewhere. White found slight diminution of elastica tissue in the deep layers of the corium in cutaneous amyloidosis. In pingueculae Fuchs described skeins and clusters in which the elastic fibrils were so delicate that the aggregates resembled homogeneous masses. Here there was definite degeneration of networks.

An important group of cutaneous lesions, described as xanthomatous in nature, are characterized by degeneration of the elastica, with formation of clumps and skeins of fibrils. Darier segregated a group in which there were similar pathologic changes. He gave this entity the name "pseudoxanthoma elasticum." Since his original description other names such as "elastoma" and "elastic naevus" have been applied to the condition. This disease usually is found in young people and often is familial. The primary lesions tend to be papular and symmetrically distributed. They appear chiefly in the skin of the unexposed parts of the body, especially in the regions of articular folds. Here, the skin may become greatly pendulous. Darier described an increase in the elastic substance of the skin. Many fibers were thickened, basophilic, vacuolate, nodular or ruptured. Sometimes they were in clusters or in the form of scattered granules. Throne and Goodman reviewed the literature carefully. They found that the elastica in the middle and deep portions of the corium showed the greatest degeneration. This was exhibited chiefly by swelling, proliferation and fragmentation of fibers. The collagen was either normal or swollen and basophilic. Giant cells in which fragments of elastica were found were occasionally present. Calcification rarely occurred. Sternberg did not believe that the masses of "elastica" in pseudoxanthoma elasticum were of elastic tissue region. Grönblad in an excellent monograph emphasized the high incidence of "angioid streaks" of the retina in the disease. He attributed their formation to degeneration of the choroidal elastica and contended that the syndrome was a manifestation of a systemic disease of elastic tissue, affecting chiefly the skin, buccal mucosa and heart. Another site of predilection was the choroid, by the involvement of which angioid streaks of the retina were formed. Apparently the blood vessels were affected to a lesser extent.

Jones and his collaborators did not believe that the disease could be differentiated histologically from senile elastosis. They contended that the disorder should be considered as a peculiar manifestation of early cutaneous changes similar to those in senile skin. Lewis and Clayton also found that the processes were similar. Montgomery contended that in typical cases the degeneration seemed to be almost entirely confined to the elastica and that one should be able to differen-

tiate the disease from senile elastosis. He expressed the belief that the disease was the result of defective formation of elastic tissue.

A process closely akin to that of pseudoxanthoma elasticum has been described by Juliusberg. In this disease there were yellowish cutaneous lesions which resembled scars. In these areas the alterations in the elastica were similar to those in pseudoxanthoma elasticum.

In retrospect, the evaluation of certain studies of cutaneous elastica should be guided by a thorough knowledge of the normal variations in elastic tissue. Awoki found in grossly normal skin from routine autopsy material that elastic tissue showed wide variations which were within the limits of many "pathologic" changes attributed by other authors to the effects of disease.

The degeneration of the elastica which accompanies advanced age may be found not only in the skin but also in other parts of the body. In the spleen the elastica may undergo atrophy, which is especially prominent in the inner half of the capsule. Diminution of elastica is common in the sclera of aged people (Jaensch). There is a question as to the part played by atrophy of elastica in the senile type of non-obstructive pulmonary emphysema. It is well known that deterioration of the elastic tissue in arteries is an accompaniment of advance in age. These changes will be considered in greater detail elsewhere.

Mechanical Factors.—The alterations in elastica which generally are assumed to be the result of mechanical forces should not be considered as wholly secondary to physical trauma, because often in these instances there is an associated inflammatory reaction. Neither do all observers believe that the deterioration of elastica in purely inflammatory lesions is of chemical origin. Du Mesnil de Rochemont contended that the injuries were produced by chemical influences. Guttentag and Schulz believed that the changes in the elastica in all lesions, inflammatory or otherwise, are a manifestation of pressure atrophy, such as Obermüller described in instances of prolapse of the vagina and prolonged use of a pessary. These conflicting opinions have been carried into the discussions which deal with the various conditions in which there is injury of the elastica.

Thus, in relation to the vascular system, the question as to whether the disruption of elastica fibers in arterial walls is always of mechanical or inflammatory origin has been studied. Von Recklinghausen found fissures in the media of fresh miliary aneurysms. Helmstädt, Manchot and Eppinger advanced the theory that a mechanical rupture of the elastic fibers is the basic factor which leads to the formation of an aneurysm. It is well known that in many places where the arterial elastic tissue has been destroyed the constituent elements of the media have been replaced by fibrous tissue. Koester, Krafft and Znurdeeg

contended that these foci are evidence of an inflammatory process which involves all types of structures. Manchot, Thoma, Zwingmann, Schulmann and Eberhardt believed that the effects were produced fundamentally by mechanical means. Manchot demonstrated abnormal elastica at a distance from the scarred areas. Thoma emphasized the significance of altered elastica as of importance in the pathogenesis of arteriosclerosis. He found small fissures or defects in the media. These appeared to be independent of the condition of the regional elastic tissue. He believed that all elements of the arterial wall frequently are involved and that often in the region of tears in the elastic lamellae there are cicatrices and leukocytes. If only a few elastic fibers parted, it seemed that the connective tissue and smooth muscle, even though not directly involved, tended to undergo atrophy and hyaline degeneration. Jores (1902) stated that although elastic fibers are destroyed by inflammation, a traumatic injury to the wall of a blood vessel evokes abundant regeneration of elastica. Fabris, after producing necrosis of the walls of blood vessels concluded that the destruction of elastica was similar to that which previously had been considered to be of mechanical origin. Katsurada came to the conclusion that pinching the skin causes no significant changes in the elastica unless there is an inflammatory reaction.

The pathogenesis of cutaneous striae is not entirely clear. The factors which suggest a mechanical origin are as follows: the occurrence of the striae with a rapid increase in body volume, their appearance along lines of cleavage of the skin transverse to the planes of greatest tension, their development in those sites where the skin is delicate, and finally, the interpretation of the histologic changes. Brünauer found certain exceptions to the mechanical theory. The exceptions indicated that toxic or endocrine influences may play a role. The theory that there may be specific "elastotoxins" was entertained.

In early hypertrophic striae or "welts," an edema of collagen, an increase in the number and size of the nuclei of connective tissue cells, dilatation of vessels and infiltration of perivascular tissues with round cells and polymorphonuclear leukocytes are common (Ebert). The normal elastica in such striae is supplanted by a multitude of delicate, poorly-stained fibrillae arranged parallel to the surface of the skin. There is some collacin. The elastica in the atrophic striae tends to disappear, the connective tissue condenses, the inflammatory reaction subsides, and new-formed elastic fibrils may arise at the margins of the lesion.

Other instances in which abnormal stresses may affect the elastica are pulmonary stasis, emphysema of the lungs, asthma, uterine pregnancy, phlebectasia and edema. In chronic pulmonary congestion there

usually is great diminution in the amount of elastica. Jores (1902) showed that this was more apparent than real, because many fibers which remained were not readily distinguishable; they simply had lost their affinity for specific stains. Degeneration of elastic tissue and diminished elasticity of the lungs have been proposed as causes of nonobstructive emphysema of the lungs. Sudsuki found that in the nonemphysematous portions of such lungs the elastica was normal. In the emphysematous portions there were variable degrees of change. The larger fibers in the early stages of the disease seemed to exhibit the greatest degeneration. Later the delicate fibrils were involved. The deterioration of the elastica occurred no earlier than recognizable degeneration of other tissue elements. He concluded that emphysema is not caused by either deficiency or degeneration of the elastic networks. Loeschcke described hyperplasia and Orsos (1906) found evidence of regeneration of elastica in emphysematous lungs. Wawerla described widespread degeneration, calcification and iron impregnation of elastic fibers in the lungs of a patient with bronchial asthma. Pagel stated that the only alteration in bronchial asthma is an increase in the amount of elastica in medium-sized bronchi.

There are interesting changes in the pregnant uterus. Woltke, W. Pick and Iwanoff showed that there is great increase in the amount of elastica during the early stages of pregnancy. In the second half of the gestation period the fibers undergo atrophy in a manner similar to that which occurs in pathologic processes. At the end of pregnancy the diminution is so great that Davidoff and Poroschin offered this as an explanation for certain ruptures of the uterus.

Scagliosi studied the effects of stasis on veins. He concluded that the degenerative changes in the medial elastica are secondary to the degeneration of smooth muscle. He described new formation of intimal elastica adjacent to areas of medial degeneration.

Most authors agree that prolonged edema is very injurious to the elastica. The gradual disappearance of the networks in the edematous skin affects the delicate fibrils first. The degeneration progresses inward from the papillary zone and is most severe in those tissues which have the richest complement of elastica (Unna, 1896).

Little is known about the atrophy of elastica from disuse except so far as this factor may exert an influence in the pathologic states which have been considered. In this regard, however, Loeschcke described an atelectatic accessory pulmonary lobe in which the elastica had failed to develop beyond the stage found in the infant at maturity.

Of further interest are the studies on skin grafts made by Enderlen. In Thiersch grafts the elastic fibers gradually degenerate. New elastic networks develop. The new-formed fibrils appear within twenty-one

to thirty days. Fifteen to eighteen months later, the tissue again is supplied richly with elastica. The new elastic tissue does not have as orderly an arrangement as the networks of normal skin. The elastica completely degenerates in the Krause grafts, and no regeneration occurs.

Chemical Factors.—It would be presumptuous indeed to dwell at great length on alterations in the chemical environment and their possible effect on the functional integrity or morphologic aspects of elastic fibers. In the preceding pages allusions have been made to a few of the facts which are established. The atrophy and degeneration which occur locally in various inflammatory lesions and in neoplasms have been ascribed by certain authors to chemical influences. The general diminution in the functional efficiency or actual deterioration of elastic fibers in certain systemic diseases has been attributed to "toxins" for want of more accurate knowledge. In experimental vitamin C deficiency Wilton showed that there is atrophy of elastic fibers and change of elastin into elacin, thus continuing the original observations made by Rheindorf bei Tuschler. It has been learned that applications of arsenic, tar and roentgen rays to the skin of mice within certain limits stimulate local development of "resorcin" fibers (Bierich, 1922). The influence of endocrine secretions and constitutional and hereditary factors will be considered later.

Thus, although the chemical influences and the means by which they may alter the elastica are wrapped in obscurity, one knows a little more about the affinity of this tissue for certain metallic elements, namely, calcium, iron, silica, silver and lead.

The affinity of elastic tissue for calcium and iron has been recognized for a long time. These two elements may be deposited in or around the fiber. Incrustation apparently is more common than impregnation. The two elements may occur together or independently, and there is no absolute ratio between the amounts of each which may be deposited (Gierke). An injured fiber has more affinity for these metals than a normal one. However, Katase produced calcium deposits in apparently normal fibers by injecting large quantities of calcium into animals. Learner obtained similar results with an overdosage of parathyroid.¹ Köckel, Bittrolf and Davidsohn were among the first to demonstrate calcified and iron-incrusted fibers in the lungs. Hofmeister stated that calcification of the elastica in the lungs usually accompanied *Kalkmetastase*. Jores (1902) found that calcium often is deposited in and around elastic fibers in blood vessels. The elastica interna is involved most frequently. Ravault studied this distribution by micro-incineration. Ehrlich discussed fully the iron and calcium incrustation of elastic fibers, especially that in the cerebral and thyroid arteries and

1. The preparation used was equivalent to solution of parathyroid U. S. P. XI.

in old splenic infarcts. Schuppisser described several instances in which the elastic fibers had attracted large amounts of iron. Lubarsch stated that in one type of calcium and iron deposition in the spleen the distribution corresponded to that of the elastica. Davis and Warren described calcification of the elastica of the skin in diabetes. The occurrence of fibrils incrustated with calcium and iron within giant cells was described by Rona. Pelagatti and Sprecher did not accept these as elastic fibers. Rona assumed that the deposition of metals occurred after the fiber had been engulfed by the giant cell. He demonstrated these fibers in giant cells of tuberculosis and leprosy. Similar inclusions in giant cells were found in mycosis fungoides (Delbanco), in paschachurda (Ssudakewitsch) and in sarcoid-like lesions (Hektoen).

Sobotta stated that elastic cartilage undergoes calcification much less frequently than hyaline cartilage.

According to Wells, the aging of elastica enhances its affinity for silica as well as its affinity for calcium and iron.

The attraction of elastic tissue for silver was noted by Blaschko, who studied instances in which silver particles had become embedded in the skin of silver workers. He found that the silver became dissolved in the course of time and then precipitated largely on the regional elastica. In a case of silver poisoning by the alimentary route, it was only with great difficulty that he was able to demonstrate deposition in the elastic fibrils of the skin. Unna (1896) stated that silver deposits in the skin always were limited to the connective tissue, especially to the elastic fibrils and limiting membranes. Lubarsch cited an instance of argyrosis in which silver deposits in the spleen were limited to the elements of connective tissue. So far as lead poisoning is concerned, it has been stated that there is a tendency toward severe arteriosclerosis. Injections of large amounts of lead fail to disclose any specific affinity of the elastic tissue for lead (Kumita).

Tumors.—Tumors, especially if they are malignant, are usually entirely free from elastica. Elastic fibers may occur in the stroma, but they are few and usually represent the elastica of the invaded tissue which has escaped destruction by the neoplasm. Krösing and Passarge found disintegration of delicate fibrils at the margins of tumors. This has been attributed by many subsequent observers to the proliferation of connective tissue and the general inflammatory reaction. Kromayer described degeneration of fibrils in the area of cellular infiltration in the stroma of tumors. In neoplasms experimentally induced with tar, nematodes and roentgen rays similar alterations occur in the elastica (Bierich, 1922). In certain instances the "elastic" fibers in the cutis were increased, but in these cases the epithelium had not extended into the corium. As the epithelial cells invaded the corium, there was disappearance of elastica, especially in the zones adjacent to the tumor.

Various types of atrophy and degeneration of elastica may occur in association with neoplasms. The most common are simple atrophy, skein formation and clumping of fibrils. Elacin fibers also are frequent (White).

Several benign epithelial tumors have been studied for elastica. White found an abundance of elacin fibrils but no elastin fibers in senile keratoses. There are nodular accumulations and dense networks of elastica in epithelioma adenoides cysticum (W. Pick). Unna (1896) found that in epithelial cutaneous nevi, if cell nests are prominent, there is a tendency for elastica to disappear between them. He believed that this loss of elastica aided in the mechanism by which the nevus became elevated above the plane of the skin. Most interesting of all is the carcinoid or argentaffin cell tumor, in which the stroma is rich in elastic fibers, smooth muscle and nerve fibers (Masson). Bailey studied benign and metastasizing tumors of this type. Elastic tissue was abundant and comprised an integral part of the stroma, not only in the intrainestinal growths but also in the mesenteric extensions and metastases in lymph nodes. He found hyperplasia of the elastica of the adventitia of small and medium-sized arteries. The elastic fibers of the tumor seemed to be derived in part from the networks in the vascular walls. He concluded that the formation of elastic tissue fibers in considerable numbers as a part of the stroma seemed to be a unique property of argentaffinomas.

Benign tumors of mesenchymal origin are little more favorable to the development of elastic fibers than benign epithelial growths. Virchow (1858, 1889) cited instances in which new-formed elastic fibers were demonstrated in cavernoma, enchondroma, endothelioma and myxoma. Karrenstein described elastic fibrils in myxoma of the heart and mentioned several similar tumors that he collected from the literature. Unna (1896) stated that there is little or no elastica in cutaneous fibroma. He found absence of elastic tissue in angioma and believed that the lack of elastica in the hypertrophied venous channels of the angiomatous nevus offered an important criterion by which the vessels could be distinguished from normal veins. White noted that elastic tissue is scanty in cutaneous leiomyoma. On the contrary, elastic networks frequently occur in uterine leiomyoma.

Very few studies of elastic tissue in malignant tumors have been reported. Mention has been made concerning malignant carcinoid or argentaffinoma of the alimentary tract. It is well known that carcinoma of the skin quickly destroys elastic tissue. Zieler concluded that the degeneration occurred in the zone of infiltration at the periphery of the tumor. He found that the destruction of the elastica

is greater if the zone of infiltration is advancing rapidly. Degeneration of elastic tissue without evidence of regeneration occurs in gastric carcinoma (Inouye). New formation of elastic tissue rarely has been described in the stroma of cutaneous and mammary carcinoma (Virchow, 1858). White found that the elastica was either reduced or absent in cutaneous sarcoma. Virchow (1858) cited instances in which there was apparent new formation of elastic fibrils in fibrosarcoma. The areas of infiltration of the skin in mycosis fungoides show either great reduction or complete disappearance of elastic networks (Jores, 1902; White). Delbanco found inclusions which were presumed to be fragments of elastic fibrils in the giant cells of mycosis fungoides. In this regard it may be added that von Hansemann and Zieler described fragments of elastica in the cytoplasm of various types of tumor cells.

So far as I am aware there is no tumor which is characterized principally by formation of elastic tissue. It seems that such tumors must occur, because the elastic fiber as to its origin has many features in common with the collagen fiber. Tumors which form an abundance of collagen are frequent. It has been suggested that pseudoxanthoma elasticum is essentially neoplastic, but there is no good reason for supporting this contention.

Congenital, Hereditary and Endocrine Diseases.—The influence of the endocrine glands on the formation and maintenance of the bodily tissues often is bound intimately with what one may choose to call constitutional, congenital and hereditary states. Therefore it seemed reasonable to consider these subjects at the same time.

Most authorities believe that the disease pseudoxanthoma elasticum is a manifestation of hereditarily defective elastic tissue. This malady, which is characterized principally by a peculiar degeneration of the cutaneous elastica and by angioid streaks in the retina, has been discussed briefly in the preceding pages.

Epidermolysis bullosa hereditaria is a rare congenital disease in which vesicles and bullae form in any part of the integument or mucous membranes subjected to traumatism. In this disease Engman and Mook described absence of elastic tissue in the papillary and subpapillary regions of the grossly normal derma. The elastic tissue in the deeper layers of the cutis was scanty and structurally defective. Other workers have corroborated their observations. This abnormality of the skin has the hereditary transmissibility characteristic of a mendelian dominant, affecting both sexes in almost equal numbers.

The constitutional bodily type designated as the asthenic habitus is due, according to Payr, to a low value of contractile and elastic elements. He claimed that patients of this type have an increased tendency toward overgrowth of connective tissue in the process of repair. No careful

comparative histologic studies have been made of the tissues of various constitutional types, but if such studies are ever made, the results should be of interest (Bauer). Sudsuki stated that there is wide variation in the amount of elastica in the lungs of normal people. He made no attempt to correlate his observations.

If elastic tissue has a part in the maintenance of elastic tone in the skin, it is reasonable to assume that among various persons there may be not only quantitative but also qualitative differences of this tissue. In this regard the "india rubber men," classic sideshow attractions, may be mentioned. These persons have abnormally extensible skin, which is of such a nature that the condition is called *cutis hyperelastica*. There is little change in the histologic appearance of the elastic network. The fibrils exhibit greater tortuosity, angulation and spiral winding than normal, but the principal abnormality seems to be in the form of split collagenous fibrils (Unna, 1896).

In treating of the influence of the endocrine secretions, one must consider the normal and abnormal imbalances of glandular activity. The remarkable changes in the elastic tissue of the uterus and its arteries during pregnancy have been described in the preceding pages. It does not seem reasonable to believe that chance abetted by mechanical forces could bring about such cyclic changes as those which occur in the elastica of ovarian vessels in the course of menstruation and ovulation. Furthermore, it is generally accepted that the development of *striae gravidarum* cannot be explained by any theory which rests solely on a mechanical or an inflammatory basis. Of greater interest is the fact that hypertrophic *striae distensae* occasionally appear in girls at puberty even though there is no unusually rapid gain in weight (Ebert). This may be linked in some fashion with the Cushing syndrome, pituitary basophilism (Cushing). In this syndrome, which is related in some way to basophilic cell adenoma of the hypophysis, the presence of purplish cutaneous *striae* in men as well as in women is one of the cardinal features. Dr. Merrill Sosman called my attention to a patient with this syndrome who has received roentgen treatment of the pituitary. She has shown a remarkable constitutional improvement, and the purplish *striae* have become pale and atrophic. Purplish *striae* also have been described in patients with carcinoma of the adrenal.

The elastica as affected by other endocrine disorders has received scant attention. Unna (1896) described hypertrophy of collagen without alteration of the cutaneous elastica in acromegaly. In myxedematous skin he found many basophilic fibrils and some evidence of multiplication of the elastica, although much of the connective tissue framework of the skin had degenerated. Foerster described degeneration of elastic and collagenous tissue in myxedema. The elastic fibrils were swollen,

and the collagen tended to disappear. Both elements were often fused into homogeneous hyaline masses. Reuter found mild degeneration of collagenous and elastic tissue. This was independent of edema and occurred when the basal metabolic rate was low. He stated that the histologic changes were similar to those which occur in solid edema of the extremities of patients with exophthalmic goiter. In diabetes the frequency of arteriosclerosis with attendant deterioration of the elastica of vessels is well known. Davis and Warren described hyalinization and calcification of cutaneous elastic fibers in diabetes. Orsos (1926), described deterioration of elastica in the lymph nodes of 2 diabetic patients.

Thus it seems that the nature of the elastica of the body may be influenced by congenital and hereditary factors and that in normal persons and in persons of certain constitutional types, as well as in those in various states of endocrine activity, the elastica may exhibit important variations of local or general distribution and of qualitative or quantitative nature.

SUMMARY

One hundred years have passed since elastica was identified as an elementary tissue. The belated segregation of this tissue is to be attributed in part to the fact that it is always accompanied by collagen in the mammalian body and is often associated with smooth muscle. The tissue is composed of continuous fibrils, bands and membranes. It is especially abundant in systems which undergo considerable fluctuations in size, particularly if the deformations are rhythmic.

The sequences in the formation of the elastica in the developing embryo indicate that, although the structure and distribution of elastic tissue may in part be predetermined, functional demands beginning in early embryonic life also play an important role. The maturation of elastic tissue in the embryo with respect to acquisition of form and characteristic staining reaction is a relatively slow process. This is also true in the evolution of the tissue from a phylogenetic point of view. It seems to have no important place in the structure of invertebrates and lower vertebrates, achieving full significance only in the higher vertebrates.

The histogenesis of the elastica is obscure, even though we accept a cellular theory of its origin and the broad postulate that it arises through a continuous change of endoplasm into ectoplasm. Presumably, the fibroblast is implicated in the formation of elastic tissue. If this is true, it is certain that the mechanism for the formation of the elastica is not influenced by stimuli in the same way as the mechanism for the formation of collagen.

The tissue has the physical properties by which bodies achieve high elasticity in the common sense of the term. Within the elastic limits it has great extensibility and an inherent capacity to return promptly to its original shape after the deforming force has been removed. It is not known whether any elastic fibril freed from encumbrances obeys the laws of elasticity as applied to rods and wires.

There is no proof that all structures which are classified as parts of the elastica are chemically identical. However, those elastic tissues which have been studied seem to have similar chemical properties. They are highly resistant to the action of ordinary laboratory reagents and belong to the heterogeneous class of scleroproteins. Mature elastica has high reducing power and is more acid than collagen, with which it is often associated. These properties serve as a basis for differential staining reactions.

The characteristic staining reactions are subject to wide variations. They are acquired slowly in the embryologic, phylogenetic and in vitro formation of the tissue. A change in the important physical properties or in the chemical structure of the tissue is not necessarily accompanied by a change in the morphologic aspects or in the staining reactions of the tissue. Conversely, an alteration in the staining reactions does not always signify an important change in the functional capacity or in the structural integrity of the tissue.

The tissue is of importance from the physiologic point of view. It is designed for the mechanical coordination of movement of separate units of structures with which it is associated. It aids in the dissemination of stresses which are directed at isolated points. It conserves muscular energy through partial maintenance of tone during the phase of relaxation of muscular elements. It serves as a restraining bulwark against the possible injury of excessive forces. It aids materially in the return of an organ or tissue to its natural shape after the deforming force ceases to act. Its function is always influenced not only by its inherent elastic qualities but also by the elasticity of the tissues with which it is intimately bound.

The morphologic changes which the tissue undergoes in various pathologic states have been studied thoroughly. No attempts have been made to correlate accurately the loss in the functional capacity of the tissue with changes either in morphologic appearance or in staining reactions. However, it is generally recognized that the accepted evidences of degeneration do not always accompany diminution in the functional capacity of the tissue. Degenerative changes may go to completion or be interrupted at various stages in the usual sequence of atrophy and disintegration. If degeneration has progressed to a measurable degree from a morphologic standpoint, there is limited capacity of the

tissue to regain its structural integrity. Regeneration of the tissue does occur occasionally, and fibrils may form even in regions where none was present normally. However, once the elastica in a region has disintegrated under pathologic circumstances, the replacement is usually meager and imperfect. If the normal architecture of the tissue has not been greatly disturbed in the presence of degeneration of the elastica and no inflammatory reaction has accompanied the deterioration, a fairly perfect regeneration of the elastica sometimes occurs.

Inflammatory lesions are notably injurious to the tissue, irrespective of the cause of the inflammatory reaction. Suppurative processes of acute or chronic nature, especially if they are accompanied by an excessive production of granulation tissue, are particularly deleterious. Necrosis of tissue in the absence of suppuration often permits an unusually perfect retention of the morphologic character of the tissue.

There are many conditions in which the elasticity of the fibers seems to be reduced in the absence of any significant morphologic change. These are much too numerous to mention here, but it is to be emphasized that disturbances in the functional capacity may be temporary. The function and morphologic character of the elastica have not been adequately studied in the various disorders due to vitamin deficiencies. In vitamin C deficiency there is some evidence of alteration in the staining reaction of the tissue. There are no data which are concerned with the functional capacity of the tissues in any of the deficiency diseases.

Neoplasms, especially if they are malignant, are usually free from elastic tissue even though there may be an abundant collagenous stroma. The only carcinoma which is accompanied by a significant amount of newly formed elastica is the argentaffinoma. No sarcoma has been described in which there was any evidence that the neoplastic cells had been instrumental in the production of elastic tissue. Benign cutaneous tumors have been described in which there was a large amount of poorly formed and degenerating elastic tissue. The malady in which these tumors were observed to occur is known as pseudoxanthoma elasticum; it is often characterized by angioid streaks in the retina.

There is some evidence that heredity may exert some influence on the quality and content of the bodily elastica. Elasticity of tissues varies among different persons. Hypoextensible and hyperextensible arteries occur in apparently normal subjects. Minor differences among comparable persons in the elasticity of the skin, dating apparently from early life, are common observations. An extraordinary elasticity of the skin is occasionally present in a disease which is known as cutis hyperelastica. Evidence of defective formation of elastica in the skin is the most important pathologic feature in a hereditary disease which is known as epidermolysis bullosa hereditaria.

There is good evidence that various states of activity of some endocrine glands are accompanied by alterations in the elastica. In diabetes there is commonly deterioration of elastic tissue. Perhaps the most outstanding example of the possible influence of endocrine secretions is the alteration in the elastica of ovarian vessels in the menstrual cycle. The increase of elastica in the uterine wall in the early stages of pregnancy and its rapid disappearance in the latter weeks of gestation are not explicable by the usual factors which bring about deterioration of the tissue. This is perhaps less true of the involutional changes in the walls of uterine arteries after the termination of pregnancy or in the walls of placental arteries as the period of gestation nears its termination.

The most important influences on the integrity of the elastic tissue are in some way related to age. The tissue increases in amount in the various organs over a period of several years. Then, beginning in the second decade of life, there is gradual diminution in the elasticity of the tissue. This loss of elasticity is a generalized involvement of the elastic networks, whether they are in the arteries, lungs, skin or elsewhere. In the age period between 10 and 60 years the arterial elasticity is reduced to approximately one half of that optimum which existed in the early years of life. This is independent of the atherosclerotic process. Accompaniments of the gradual decay are impregnation with calcium salts, loss of characteristic staining reactions, morphologic disintegration, and relaxation of the structures normally supported by the elastica. Thus, a large part of the visible effects of age—the functional effects in all instances and the morphologic in the majority of instances—seems to be attributable to this inexplicable deterioration of the elastica. It is particularly interesting that in placental arteries sequences identical to those described in the aging process as it affects the arteries of the average person follow in rapid succession, taking place in the short span of a few months.

In conclusion it is hoped that this survey will direct attention to a much neglected tissue. It is fair to state that no more than a beginning has been made in the study of this tissue. It may be permissible to point out that an analysis of the data indicates that future investigators may profitably concern themselves with a substance or substances which are elaborated from relatively simple chemical compounds and deposited in elective sites. It may be suggested that some of the substances from which the tissue is constructed are capable of maintaining a metabolic interrelationship with the components of the medium in which they persist. It may be predicted that these substances are of such labile nature as to react reversibly *in situ* and to be amenable to resorption, redeposition and augmentation in response to influences of either a local or a general nature.

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Notes and News

University News, Promotions, Resignations, Appointments, Deaths, Etc.—Victor H. Bassett, health officer of Savannah, Ga., and at one time professor of pathology in Milwaukee, died Nov. 3, 1938, aged 67 years.

L. Corsan Reid has been promoted to the rank of assistant professor of pathology in the New York Medical College.

Thomas Francis Jr., member of the staff of the International Health Division of the Rockefeller Foundation, has been appointed professor of bacteriology in the New York University College of Medicine.

Society News.—The annual meeting of the American Association of Pathologists and Bacteriologists will be held at the Medical College of Virginia, Richmond, Thursday and Friday, April 6 and 7, 1939. The headquarters will be Hotel Jefferson. The secretary is H. T. Karsner, 2085 Adelbert Road, Cleveland.

The sixty-eighth annual meeting of the American Public Health Association will be held in Pittsburgh, Oct. 17 to 20, 1939, with headquarters at the William Penn Hotel.

The fiftieth annual meeting of the Association of American Medical Colleges will be held at Cincinnati, Oct. 23, 24 and 25, 1939.

The American Board of Pathology announces that it has changed the date of its next examination from April 8 to 9 to April 3 to 4.

The Scandinavian-American Foundation is interested in an exchange of medical students between Scandinavian countries and the United States. The Association of American Medical Colleges will cooperate.

CORRECTION

In the article by Dr. Harold Wood entitled "A Tray for Dehydration, Clearing and Paraffin Impregnation of Large Quantities of Tissue," in the August issue (ARCH. PATH. 26:532, 1938), "75" should be substituted for "225" in the fourth line of the first paragraph under the center head "Description of Tray."

Obituaries

HERBERT U. WILLIAMS, M.D.

1866-1938

Dr. Herbert U. Williams retired from teaching four years ago. He used to spend the winter months in warmer climates, in central and southern states, in the leisurely pursuit of his pathologic studies of ancient diseases. Again he was planning to go south, for he enjoyed excellent health until very recently. He was hardly aware of harboring an organic lesion when, late in the evening of December 8, last, while greeting friends at his favorite club, he was suddenly seized with a heart attack. Shortly after, still completely unconscious, he died at the Buffalo General Hospital, the hospital in which his career had started. Postmortem examination revealed marked sclerotic aortic stenosis, with distinct narrowing of the coronary ostia, recent pulmonary edema and marked dilatation of the stomach, with constriction from chronic ulcer at the pyloric ring.

Williams, who was a native of Buffalo, was born Nov. 28, 1866. He graduated from the old Central High School, going from there to the University of Michigan for two years. In 1886 he entered the medical school of the University of Buffalo and obtained his medical degree in 1889. He continued his medical studies at the University of Pennsylvania, where he obtained a doctorate in 1891. Postgraduate studies then took him to the College of Physicians and Surgeons in New York, to the Johns Hopkins Hospital (in the department of William Welch) and to the Pathologic Institute of Johannes Orth in Göttingen, Germany. In 1894 Williams was appointed professor of pathology and bacteriology at the University of Buffalo; soon after, he became head of the department. In this capacity he served the medical school for forty years, until his retirement in 1934. From 1912 to 1915 he was dean of the medical school.

The early scientific papers of Williams concerned the nature of fat necrosis—its morbid anatomy, causation and experimental production. In the field of experimental pathology Williams was one of the early pioneers. By ligating the pancreas in cats, he produced marked fat necrosis. However, the positive results were largely enhanced by infection, and further experiments were carried out to eliminate the role of infection. Pancreatic tissue was inserted in the subcutis of cats by means of a special glass cannula. He was then able to produce fat necrosis in a number of instances in which infection was eliminated.

The histologic changes were carefully analyzed and described. These and other experiments verified the conclusion of Hildebrand that the mere action of the ferments of the pancreas brings about fat necrosis. In his control studies Williams found that fat necrosis occurred also spontaneously in different animals. Other experiments dealt with the causes and genesis of acute pancreatitis. In these he placed in the bile ducts glass cones, which he pushed through the common duct in imita-



HERBERT U. WILLIAMS, M.D.
1866-1938

tion of the passage of gallstones. Then he injected duodenal content into the pancreatic duct through a blunt cannula. By these methods he produced acute necrosis of the pancreas in a few cases and concluded that regurgitation of the duodenal content in the diverticulum of Vater, the way having been opened by the passage of stones, leads to the entrance of enterokinase into the pancreas, resulting in digestion of pancreatic tissue and necrosis. He was unable to produce these effects with an injection of sterile enterokinase alone. In another paper he

stressed the role of traumatism in necrosis of the pancreas following operation on the gallbladder.

Williams also studied the new formation of elastic fibers in the stroma of carcinoma at the time when the Weigert stain had just become known. In his paper on this subject, as well as in a critical review of the literature on plasma cells and mast cells, it is evident that he had a considerable command of technical methods. Much of his description of the granules in mast cells, observed after the use of different fixatives, is based on his own experience. Other, mostly morphologic studies concern the histologic changes in the sweat glands in chronic nephritis, the incidence of trichinosis in routine postmortem material and the lymphomatous tumors or tumor-like nodes in the normal dog's spleen. In the latter instance he made a systematic investigation of the spleens of 720 dogs. A few brief papers of bacteriologic interest might be mentioned: one concerned a mixed Welch bacillus-Streptococcus infection causing pyelitis; a second, results of attempts to cultivate trypanosomes from frogs; a third, sputum examination during the influenza epidemic, and a fourth, experimental studies of the agglutination of human erythrocytes by horse serum. He also wrote a manual of bacteriology, mainly for the use of medical students, which had five editions.

In the years following the great war Williams became increasingly interested in the history and antiquity of diseases, especially in the history of syphilis. To the readers of the *ARCHIVES OF PATHOLOGY* his reviews on paleopathologic topics and on the origin and antiquity of syphilis are well known. These studies brought Williams a world-wide reputation among pathologists, medical historians and anthropologists alike. In the field of human paleopathology he published original observations on a peculiar symmetric osteoporosis which he had found chiefly in ancient skulls from Indian children. These he found especially in the areas where the cultivation of Indian corn was most highly developed. He felt that this osteoporosis was possibly allied to rickets or scurvy or some types of anemia in children, as the effect of a lack of vitamins or of other dietary deficiency. It had not been found in skulls of white people. This review included diseases known in modern times—for instance, tuberculous bone lesions in a neolithic skeleton (from Europe) and in a mummy of a priest of Amen. He examined two Peruvian mummies and found distinct evidence of arteriosclerosis with a calcified thrombus. Deforming arthritis, he found, was apparently common among ancient people, as well as biliary and urinary calculi, cirrhosis of the liver and small osteomas; however, according to Williams, dental caries was less common in ancient times and became more frequent with the alteration of living conditions in modern civilization.

In his review of the origin and antiquity of syphilis Williams not only analyzed critically all reported cases of ancient syphilis of the bone, many of which he had studied personally in the large museums of Europe and America, but he added entirely new material from the American continent. He gave an excellent description of syphilitic lesions of the skull and long bones and of the difference between these and the lesions of Paget's disease. In his opinion, careful examination with the unaided eye, especially of the long bones sawed lengthwise, gave more information than any other method. Many ancient bones showing periosteal new growth were examined histologically.

As Williams had traveled a great deal and had visited many museums of pathology both here and abroad, he had a large material at hand for a comparison of pre-Columbian skulls, such as that in the famous Pecos case, with skulls from cases proved to be instances of syphilis, at the museum in Prague. Study of a very large material of ancient skulls and long bones, which was collected in different parts of the Americas, especially New Mexico, Peru, Argentina, Florida, Ohio, Tennessee and Louisiana, led Williams to conclude that the Indians were afflicted with syphilis before the arrival of the white man. It was his belief that no similar proof from the Eurasian continent had been produced; no convincing case of syphilis in Europe can be dated previous to 1500. In further proof of the American origin of syphilis Williams made citations from early Spanish authors concerning "bubas" as collected by Montejó y Robledo. From all these presentations it seems evident that syphilis was not known in Europe prior to the discovery of America or to a few years preceding that time, when the Portuguese were exploring the coast of Africa. It was apparently the question how far bone lesions in yaws might simulate those of syphilis, and its bearing on the analysis of ancient bones, which induced Williams to study yaws in various tropical islands—Haiti, Java, the Philippines. He found, however, very few evidences of bone diseases in yaws. In his review of yaws and syphilis he gave a complete histologic analysis of the primary and secondary stages in yaws and syphilis, stressing in the former especially the marked proliferation of the epithelium and the large number of leukocytes penetrating the epithelium, along with but slight involvement of blood vessels. He did not find any evidence that internal viscera were infected in yaws.

Williams was an excellent teacher, beloved and esteemed by his students through the forty years of his teaching. He tried to coordinate the teaching of pathology with that of the major medical disciplines. The University of Buffalo owes him a large debt of gratitude, not only for this, but because it was largely due to his efforts that the school of arts and sciences was successfully inaugurated in 1913, when Williams was dean of the medical school.

Williams was a member and former president of the American Association of Pathologists and Bacteriologists and a member of the American Association of Physical Anthropologists and of the American Anthropological Society. He was a fellow of the American Association for the Advancement of Science.

It was in the dark corridors of the German Pathological Institute in Prague that I first met Dr. Herbert U. Williams, about twelve years ago. He was on one of his scientific excursions, visiting the museums of the pathologic institutes in Paris, Berlin, Vienna and Prague. His kind manner and most vital interest in his scientific objectives, his seemingly timeless enthusiasm, made on all of the younger men an unforgettable impression. And so he will be remembered by countless students, the colleagues whom he helped and his fellow pathologists, here and abroad, for his teaching, his remarkable personality and straightforward character, and for his scientific work.

KORNEL TERPLAN.

GEORGE BURGESS MAGRATH, M.D.

1870—1938

George Burgess Magrath, born Oct. 2, 1870, was the only son of Rev. John Thomas and Sarah Jane (née Herrick) Magrath, natives of Maine. The former was graduated from Bowdoin College.

Born in Jackson, Mich., he moved with his parents in 1877 to Battle Creek, in 1879 to a suburb of Philadelphia, in 1882 to Hyde Park, Mass., and in 1890 to Milton, Mass., with the changing of pastorates.

In Hyde Park he went to high school three years and studied the organ under John A. Preston. Later he became the organist at the Church of the Holy Spirit, which was his father's charge.

While living in Milton, he went to the Roxbury Latin School and graduated at the head of his class, although he entered six weeks late.

He entered Harvard University in 1890. He graduated in 1894 magna cum laude, with the degree of Bachelor of Arts, and was speaker of his class. While here he became identified with athletics, especially with rowing and sculling. He joined the Union Boat Club in 1898 and from 1899 to 1917 he rowed every year on club crews. In 1907 he was on the winning senior double scull shell crew of the club in the July Fourth Regatta, and it was a familiar sight to see him pulling an oar every day in a shell on the Charles River.

In 1898 he graduated from Harvard Medical School cum laude, but before he graduated (January 1898) he worked at the Boston City Hospital in the pathologic department, under Councilman's direction, until October 1898. Then he became one of Councilman's assistants in the pathologic department of the Harvard Medical School, and there under a Bullard Fellowship he completed a research study which gained him the degree of Master of Arts in 1899. After that came concurrent appointments at the Carney, Long Island, St. Elizabeth's, Cambridge and Faulkner hospitals, where he was able to "give some help in developing the pathological laboratory as a necessary adjunct to the modern hospital."

During this time an epidemic of smallpox occurred, and in the work on this Dr. Magrath "participated," completing his work in 1903 and announcing the discovery of *Cytorrhcytes variolae* in his joint study on the pathologic nature and etiologic factors of variola and of vaccinia with W. R. Brinkerhoff and I. R. Bancroft. A collection of 28 care-

fully prepared specimens of skin from persons involved in the epidemic are to be found in the Warren Anatomical Museum of Harvard Medical School and attest the active postmortem study of the disease at that period. One recognition of this work was an offer of a professorship in pathology at Washington University, St. Louis, but this he



George Burgess Magrath

1870—1938

declined. Continuing in the department of pathology and becoming assistant to the secretary of the Massachusetts State Board of Health, he investigated outbreaks of communicable diseases.

In 1905 he changed from being assistant in pathology to that of assistant in hygiene at the Harvard Medical School and in 1907 was

selected to follow Dr. Francis A. Harris as medical examiner for Suffolk County by the then Governor Curtis Guild. Dr. Magrath expressed himself in regard to this office at the time as follows:

The duties of this office consist chiefly in the investigation of deaths due to injury of any sort and of those which are sudden or unexplained; they necessarily include service from time to time in court. The position is one affording wide opportunity for use in the service of the state of the knowledge and technical skill yielded by special training and experience. In doing my work I have sought to apply to the branch of state medicine, which my office represents, the result of the generous type of scientific medical education which it was my good fortune to receive. Since 1907, I have been appointed instructor in legal medicine. Teaching in this subject is by lecture and by demonstrations to members of the third and of the fourth year classes of the medical school. The general standard of medical jurisprudence in this country is none too high and it is my aim to help raise its level by applying to my own work the principles and the methods of modern scientific medicine and by impressing on the student the importance of the responsibility of the physician in all matters wherein medicine is brought into the service of the law.

Dr. Magrath was able to interpret General Laws, chapter 38, Medical Examiners, which was written in a vague and general manner, for the best interests of the situations he was called on to meet.

He labored with all forces to establish rules of practice which would extend the provisions of this law. These rules have been variously adopted and have become customary.

In 1908 and in 1910 he made short trips to England, France and South America. In 1914 and 1915 he assisted in the framing of legislation and in creating for the City of Greater New York a system of medicolegal inquiry based on the plan of that which he developed in Suffolk County, Mass., and he was invited to be a candidate for the position of Medical Examiner but refused.

He said: "Outside of official and academic duty I am from time to time engaged as medico-legal pathologist or consultant in matters medico-legal and in this capacity I have participated in numerous murder trials, some of them in other states. In such instances I serve only at the call of the government."

It is to be remembered that with all this fatiguing industry he was as active as ever in directing societies and clubs, in rowing and in music, his diversions.

In the World War, while desiring to go, he felt his service was urgently needed at his own "post of duty," but he did accept in 1918 the commission of Major in the Medical Corps, Massachusetts State Guard. He was "prepared to respond with my assistants and ambulance service, to call from Acting Chief Surgeon, Massachusetts State Guard, in the event of any disaster involving loss of life."

From 1919 on, his activities increased in the matter of participation in investigations of mysterious deaths. How extensive these were will be remembered by those who attended his lectures to the students or his Lowell Lectures in 1932, or heard addresses given by him before the Massachusetts Medico-Legal Society. A postmortem examination skillfully and painstakingly done with double measurements, metric and linear, was only one part of such duty. *"One must investigate the premises, know all the circumstances, use judgment and common sense, and in the pursuit of the truth be relentless."*

In 1931 a chair of medicolegal medicine was endowed at the Harvard Medical School and he was made professor at that time, having previously been instructor in the subject since 1907.

In 1935 he resigned from the office of Medical Examiner and in 1937 was made emeritus professor of medicolegal medicine at the Harvard Medical School.

An unfortunate infection of both his hands crippled his action and reduced his activities, but he filled his new leisure with precautions for his health and with enjoyment of his varied interests, particularly that of music. Indeed, he was preparing to attend a concert when he fell ill, on Dec. 11, 1938, with the cerebellar hemorrhage from which he died.

MYRTELLE M. CANAVAN.

Abstracts from Current Literature

TO SAVE SPACE THE ORIGINAL TITLES OF ABSTRACTED ARTICLES SOMETIMES ARE SHORTENED

Experimental Pathology and Pathologic Physiology

PATHOLOGIC CHANGES PRODUCED BY GASTRECTOMY IN YOUNG SWINE. S. PETRI, F. NØRGAARD and J. BING, *Am. J. M. Sc.* **195:717**, 1938.

In 6 week old pigs operative removal of the stomach gave rise to a number of severe clinical and pathologic anatomic changes that may be interpreted as similar to those found in human pellagra. Conspicuous features in this condition were arrest of growth, hypochromic microcytic anemia and extensive changes in the skin and in the central nervous system. In addition, there were diverse inconstant changes, as hyperproteinemia, plasma cell metaplasia of the spleen and lymph glands, cirrhosis of the liver and osteoporosis. In principle, the authors state, these results correspond to their observations in studies on pups, suggesting the presence of a specific factor in the stomach of swine, dogs and man which is required to maintain the skin and central nervous system in a normal healthy state.

FROM AUTHORS' SUMMARY.

EXPERIMENTAL RICKETS. G. S. DODDS and H. C. CAMERON, *Am. J. Path.* **14:273**, 1938.

Rickets was produced by the Steenbock-Block diet; healing was brought about mainly by administration of viosterol. Healing is described in detail from study of sections stained with hematoxylin and eosin, silver preparations and roentgenograms which were made frequently. The twelve roentgenologic stages of Bourdillon were used as a scale for measuring the progress of healing. The first indication of healing was calcification in the rachitic metaphysis, close to the edge of the epiphysial cartilage, whence it spread, first through the metaphysis toward the shaft and later into the cartilage toward the end of the bone. The preliminary reorganization of the metaphysis involved: provisional dilute calcification of the osteoid; calcification of the exposed cartilage trabeculae; increase in the amount of marrow, and restoration of the marrow structure to normal. These changes restored the configuration of the trabeculae to what was almost normal by exposing the rather parallel cartilage trabeculae. The end of the shaft underwent much the same changes. At the same time the projecting cartilage masses became calcified and were partly removed, leaving calcified trabeculae very similar to those in the metaphysis. During the latter half of healing the shape of the bone became corrected; hematogenic marrow formed in the new end of the shaft, which had been formed from the metaphysial region; cupping was corrected, the entire epiphysial cartilage became normal in thickness and structure, and the reorganized trabeculae attained normal dense calcification.

FROM AUTHORS' SUMMARY.

EXPERIMENTAL BRONCHIECTASIS. W. E. ADAMS and L. ESCUDERO, *Tubercle* **19:351**, 1938.

Bronchiectasis was produced experimentally in dogs. Two factors were necessary for its production, namely, incomplete bronchial obstruction and infection of the bronchi. It was produced by both aerobic and anaerobic pyogenic organisms, by tubercle bacilli and by the organisms causing distemper. Incomplete obstruction was essential; at no time did bronchiectasis follow complete bronchial obstruction. An increase in intrapleural negative pressure was important only so far as the

degree of severity of the pathologic changes was concerned; when it was present, bronchiectasis developed to a somewhat marked degree. Thus, in the explanation of this condition, the original theory of Laënnec appears to be the one most nearly correct.

H. J. CORPER.

THE GROWTH OF HUMAN BONE MARROW IN TISSUE CULTURE. G. WEITZMANN and EVA POSERN, *Virchows Arch. f. path. Anat.* **299**:458, 1937.

The technic of the culture of human hemopoietic tissues was described by Meier, Posern and Weitzmann in the preceding issue of *Virchows Archiv* (previously abstracted). The present authors proceed to the description of the growth and differentiation of a single cell type in their cultures. It has been found possible to grow marrow removed by sternal puncture, thus greatly broadening the scope of the study of marrow. The marrow of patients with chronic or cachectic disease grows more slowly than the marrow of normal persons or that of persons with acute disease. In the growth and differentiation of the cell type selected for study, four stages are described: In the first stage, that of emigration of cells from the explant into the peripheral growing zone, there are seen round, oval or polygonal cells that differ from blood cells in the finely granular and dark character of their cytoplasm. The granules give a lipoid-staining reaction with Nile blue sulfate; they are not the result of degeneration but represent lipoids formed in the intermediary metabolism of the cells. In the second stage the granulation of the cytoplasm disappears, the cells grow into giant cells, twenty to fifty times the size of the myeloid cells among which they lie, and the latter are filled with phagocytosed cells. In the third stage the cells become smaller, elongated and mononucleated, and phagocytosis is less active. From the poles of the cells processes grow out, which unite with those of other cells to form a wide-meshed network. The fourth stage is characterized by formation of fibrils. The cells whose life history has been thus followed in tissue culture are derived from the reticulum cells of the bone marrow.

O. T. SCHULTZ.

EXPERIMENTAL ALLERGIC HYPERERGIC ARTERITIS. W. RINTELEN, *Virchows Arch. f. path. Anat.* **299**:629, 1937.

Rabbits were sensitized by repeated subcutaneous injections of horse or swine serum. In the sensitized animals and in controls the carotid artery was exposed and doubly ligated under ethyl carbamate (urethane) and ether anesthesia, and the provocative dose of the antigen was injected into the lumen of the artery between the two ligatures. Within four hours fibrinoid swelling of the wall of the vessel was evident in the sensitized animals. This was followed by leukocytic infiltration. By the second day there was a marked histiocytic reaction. The inflammatory process involved the entire wall. The process usually ended in thrombosis of the artery, with replacement of smooth muscle and elastic tissue by fibrous tissue. In control animals the injection of serum was followed by only a slight perivascular inflammatory reaction, resulting from the trauma. Normal horse serum from a commercial concern did not produce hyperergic arteritis such as that described.

O. T. SCHULTZ.

Pathologic Anatomy

EXPERIMENTAL PNEUMOCOCCUS INFECTION IN TUBERCULOUS RABBITS. J. WEISSFEILER, E. N. MOROZOVA and A. I. STRUKOV, *Am. Rev. Tuberc.* **37**:93, 1938.

Rabbits infected intratracheally with virulent strains of tubercle bacilli of bovine type cope with an intravenous or an intraperitoneal inoculation of pneumococci of type I as well as control animals. The cellular reaction in a tuberculous animal clearly differs from that in a healthy rabbit. The reaction of the tuberculous animal takes a slower course, has a more exudative character and tends

more toward necrosis than the reaction of the healthy rabbit. The difference observed in the reactions of tuberculous and healthy animals can be explained by heteroallergy due to tuberculous infection. Thus, heteroallergy plays an important part in the course of nonspecific infectious processes in a tuberculous organism.

H. J. CORPER.

BUNDLE BRANCH BLOCK. W. M. YATER, *Arch. Int. Med.* **62**:1, 1938.

Bundle branch block is usually due either to disease of the coronary arteries (rheumatic or degenerative) or to hypertension resulting in strain of the left ventricle and impairment of the nutrition of the endocardium and bundle branch. Bundle branch block is as a rule associated with lesions of both bundle branches although one branch is usually more seriously affected than the other and probably determines the essential form of the electrocardiographic curve. The newer, or American, terminology is more nearly correct for bundle branch block in man, although it must be admitted that whether "right" or "left" is used to modify the term designating the diagnosis of this disturbance in conduction, the adjective merely indicates the branch more seriously affected. The uncommon form of bundle branch block, right bundle branch block, is probably due as a rule to rheumatic arteritis or rheumatic myocarditis. The common form of bundle branch block, left bundle branch block, is probably due as a rule to degenerative cardiovascular-renal disease, meaning coronary arteriosclerosis or arterial hypertension or both. A bundle branch need not be entirely destroyed at any level in order to produce electrocardiographic alterations that may be designated as typifying bundle branch block. An increased amplitude of the ventricular complex is not essential to the electrocardiographic diagnosis of bundle branch block. Any increase of the QRS interval beyond one-tenth second may indicate lesions of the bundle branches. Many questions remain unanswered in regard to bundle branch block, and many careful histologic studies must be made before most of them can be answered.

FROM AUTHOR'S SUMMARY.

PERIARTERITIS NODOSA. J. W. KERNOHAN and H. W. WOLTMAN, *Arch. Neurol. & Psychiat.* **39**:655, 1938.

Pathologic studies of 5 cases of periarteritis nodosa convinced Kernohan and Woltman that the primary lesion is in the media of the smallest arteries (periarterioles). The media first undergoes hyaline-like degeneration with necrosis, followed by changes in the adventitia and intima. The adventitia becomes infiltrated with lymphocytes, plasma cells and endothelial-like elements; the intima proliferates, causing narrowing and even obliteration of the vascular lumen. It may become separated from the media by lymphocytes, plasma cells and endothelial cells. The vascular lesions were usually present in every organ, but in a single case they were limited to the peripheral nerves; in another case, they were also very marked in the brain, and in a third case they were pronounced in the retinal blood vessels (the arterioles of the choroid). The changes in the peripheral nerves showed as swelling degeneration and complete disappearance of the myelin, followed by degeneration of the axons. In addition, there were infarcts in the nerve trunks, multiple and single. Kernohan and Woltman consider the changes in the nerves secondary to those in the blood vessels, the result of an inadequate supply of blood.

GEORGE B. HASSIN.

REGIONAL ILEITIS AND ULCERATIVE COLITIS. E. S. STAFFORD, *Bull. Johns Hopkins Hosp.* **62**:399, 1938.

Stafford discusses 10 cases in which regional ileitis, and 3 in which ulcerative colitis, was associated with lymphogranuloma. The similarity of the clinical features in these two types of cases has been noted. The typical microscopic lesion of lymphogranuloma is differentiated from the nonspecific lesions of regional

ileitis by the presence of the tubercle-like group of epithelioid cells with a central core of polymorphonuclear leukocytes. That the virus of lymphogranuloma may cause the ulcerative lesions in the colon has been considered, and the possibility suggested that the lesions of regional ileitis may also be due to the virus.

FROM AUTHOR'S SUMMARY.

VARIATION IN RETICULOCYTES OF RABBITS. P. NICOLLE, Arch. Inst. Pasteur de Tunis **27**:42, 1938.

In rabbits inoculated with classic typhus the reticulocyte count increases from 2 or 3 per hundred red cells to 6 or 7 within from ten to fourteen days.

Rabbits inoculated with *Trypanosoma congolense* present an anemia accompanied by an increase in reticulocytes. There is a rapid decrease in the red blood cell count for from eleven to nineteen days, followed by a chronic stage and eventually by a return to normal. As the red blood cells fall below 3,000,000 per cubic millimeter, the reticulocytes increase to 450,000 per cubic millimeter. There is marked leukopenia with a proportional increase in mononuclear cells. Polychromatophilia, poikilocytosis and anisocytosis are common. The anemia is one of cell destruction without permanent lesions of the hemopoietic organs.

During the period of incubation of experimental infection with *Trypanosoma equiperdum* there are successive waves of reduction of the red cell count and increase in the number of reticulocytes, with periods of return to normal. When the number of red blood cells decreases from 20 to 25 per cent that of reticulocytes increases from 50 to 100 per cent.

Spontaneous parasitic infections and trauma may also alter the unstable reticulocyte equilibrium of the rabbit.

J. B. GUNNISON.

INTRANUCLEAR FORMATION OF MELANOTIC PIGMENT. K. APITZ, Virchows Arch. f. path. Anat. **300**:89, 1937.

In a study of melanosarcoma and pigmented moles Apitz describes the formation of melanotic granules within the cell nucleus. His concept of pigment formation is that the nucleus furnishes to the cytoplasm an invisible fluid material (melanogen) which is transformed into visible pigment in the cytoplasm. In neoplasms the melanogen may be retained within the nucleus and may there be transformed into pigment. Such formation of pigment within the nucleus occurs always within vacuoles. The latter he considers characteristic of pigment-forming cells and believes that they may help determine the melanotic origin of a nonpigmented metastasis.

O. T. SCHULTZ.

MEDIASTINAL CYST LINED BY GASTRIC MUCOSA WITH PERFORATING PEPTIC ULCER. C. BOESS, Virchows Arch. f. path. Anat. **300**:166, 1937.

To 4 previously reported cases of mediastinal cyst lined by gastric mucosa the author adds a fifth. This cyst occurred in a boy $3\frac{3}{4}$ years old. It was the size of a hen's egg. A chronic peptic ulcer of the lining, which evidenced secretory activity of the mucosa, perforated into the left lung and led to death from hemorrhage. The most likely explanation of the malformation is that it originated from misplaced gastric mucosa or from an endodermal anlage of the esophagus with heterotopic potency. Also, origin from a remnant of the omphalomesenteric duct is held to be possible.

O. T. SCHULTZ.

PRIMARY THROMBOSIS OF THE DEEP VEINS OF THE LEG. R. RÖSSLE, Virchows Arch. f. path. Anat. **300**:180, 1937.

Primary autochthonous venous thrombosis is stated by textbooks to occur most frequently in the pelvic and femoral veins. Clinical evidence indicates that thrombosis of the plantar veins and of the deep veins of the leg is frequent.

While still at Basel, Switzerland, Rössle systematically investigated the deep veins of the calf of the leg in 400 consecutive necropsies in a period of five months. Of the subjects, 324 were over 20 years old; 88 of these revealed thrombosis of the deep veins of the leg. Thrombosis was frequently associated with degenerative and atrophic changes in the muscles of the calf. Rössle mentions three possibilities to explain this association: (1) that the changes in the muscles were due to the thromboses, against which is the fact that the thromboses were usually more recent than the muscle changes; (2) that degenerative changes in the muscles led to the formation of substances that caused the local thromboses, and (3) that the thromboses and muscle changes were independent of each other but resulted from a common cause.

O. T. SCHULTZ.

CHANGES IN THE CALF MUSCLES FOLLOWING VENOUS THROMBOSIS AND PROLONGED CONFINEMENT IN BED. H. VOEGT, *Virchows Arch. f. path. Anat.* **300**:190, 1937.

Rössle's observation of the frequent association of thrombosis of the deep veins of the leg with degenerative changes in the muscles of the calf led to further investigation especially from the standpoint of the effects of long confinement in bed. The muscles of the calf were examined histologically and compared with the rectus abdominus and biceps muscles. In 5 persons with venous thrombosis the calf muscles showed degenerative changes and atrophy. However, in 24 persons without thrombosis who had suffered long confinement to bed equally severe changes were observed in the calf muscles while such changes were not to be seen in the biceps and rectus muscles. Pressure, circulatory changes and inactivity are held responsible for the changes described. That the degenerating muscle tissue may liberate thrombogenic substances that lead to terminal thrombosis is admitted as a possibility.

O. T. SCHULTZ.

MASS AND SURFACE DEVELOPMENT OF THE FETAL BRAIN. H. H. MEYER, *Virchows Arch. f. path. Anat.* **300**:202, 1937.

The brains of 180 fetuses were studied between the fifth and tenth month of pregnancy as to changes in weight in reference to fetal length and as to the time of appearance and development of the more important sulci and convolutions. The time of appearance of the main sulci and the peculiarities of their development are summarized in a series of tables, the material being grouped according to weeks of duration of pregnancy as determined by fetal length. In their earliest development the sulci are bilaterally symmetric, but the symmetry is disturbed by the formation of anastomoses and other developmental changes. There is no direct correlation between the general development of the body and the development of the brain. The sulci of the brains of female fetuses revealed a greater degree of variation as the result of anastomosis between sulci than did brains of male fetuses.

O. T. SCHULTZ.

Pathologic Chemistry and Physics

COMPOSITION OF HUMAN BONE IN CHRONIC FLUORIDE POISONING. W. A. WOLFF and E. G. KERR, *Am. J. M. Sc.* **195**:493, 1938.

In a case of chronic fluoride poisoning the bones contained normal amounts of calcium, phosphorus and carbon dioxide. The fluorine content was increased up to twenty times the normal and was unequally distributed, being highest in the vertebrae and lowest in the long bones. The specific gravity of the femur was low. Data on man and animals are compared.

FROM AUTHORS' SUMMARY.

THE CEVITAMIC ACID CONTENT OF FETAL BLOOD. P. MANAHAN and N. J. EASTMAN, *Bull. Johns Hopkins Hosp.* **62**:478, 1938.

The concentration of ascorbic acid in fetal blood is regularly almost three times that in the maternal blood. Raising the concentration of ascorbic acid in the maternal blood by antepartum administration of orange juice raises concomitantly the concentration in the fetal blood, the ratio between the concentrations in the two bloods remaining similar. The high relative concentration of ascorbic acid in fetal blood suggests that the placenta exerts a selective action in respect to this vitamin.

FROM AUTHORS' SUMMARY.

THE INFLUENCE OF INFLAMMATION ON THE ABSORPTION OF SUBSTANCES OF VARIED DIFFUSIBILITY. R. G. MILLER, *J. Exper. Med.* **67**:619, 1938.

Inflammation retards the absorption of horse serum globulin and crystalline egg albumin from the peritoneal cavity and from the subcutaneous tissue, but the retardation of the absorption of crystalline egg albumin is less than that of globulin, which is less diffusible. Inflammation retards the absorption of the specific polysaccharide of *Pneumococcus* type I from the peritoneal cavity; inflammation may accelerate but does not hinder the absorption of dextrose from the peritoneal cavity. Inflammation retards the spread of trypan blue in the skin but accelerates absorption from the skin of a more diffusible dye, bromphenol blue. Phenol red is excreted in the urine with equal rapidity after injection into a normal and into an inflamed peritoneal cavity. Direct extractions of phenol red from inflamed subcutaneous sites indicate that inflammation accelerates the absorption of the dye from these areas. Inflammation retards the absorption of the indiffusible proteins, carbohydrates and dyes; it tends to accelerate the absorption of the diffusible carbohydrates and dyes.

FROM AUTHOR'S SUMMARY.

A COMPARISON OF THE INTERCHANGE OF THE BODY FLUIDS AFTER INTRAVENOUS INJECTIONS OF CRYSTALLOIDS, GUM ACACIA AND BLOOD SERUM. J. D. ROBERTSON, *Brit. J. Exper. Path.* **19**:30, 1938.

When hypertonic solutions such as 33 per cent sodium chloride or 50 per cent dextrose are injected intravenously at the rate of 5 cc. per kilogram, there is an enormous increase of the blood volume, often reaching a maximum of double the original volume. The increase takes place rapidly and only during the period of the injection. The moment the injection is stopped, the blood volume begins to fall rapidly, and in about half an hour it is normal. When 2 per cent, 0.9 per cent, 0.6 per cent and 0.3 per cent sodium chloride are injected intravenously, fluid leaves the vascular system during the injection, so that the blood volume immediately after completion of the injection is less than the theoretic level expected. After the injection the blood volume falls, but at the end of an hour it is still about 15 per cent above normal. The result of an intravenous injection of blood serum is similar to that of an injection of hypotonic, isotonic or 2 per cent sodium chloride. In the mammalian circulation sodium chloride added to acacia depresses the power of the acacia to attract fluid. When the concentration of sodium chloride exceeds 1 per cent, the attraction of fluid during the injection is greater than can be accounted for by either the acacia or the sodium chloride alone. In studying changes in the blood volume of a cat after intravenous injections of 6 per cent acacia in solutions of sodium chloride of various strengths, it was found that 6 per cent acacia in physiologic solution of sodium chloride most nearly resembles blood serum. After a hemorrhage an injection of blood serum produces in the blood volume a response similar to that after an injection of 0.9 per cent sodium chloride; and in both cases the blood volume falls rapidly after the injection. Acacia, on the other hand, produces a much slower fall in the blood volume. Acacia is retained intravascularly to a greater extent than blood serum.

FROM AUTHOR'S SUMMARY.

PANCREATIC SILICIC ACID. W. KASTEN, Frankfurt. *Ztschr. f. Path.* **50**:42, 1937.

In a series of 79 cases Kasten was unable to find any correlation between the silica content of the pancreas and diabetes, malignant tumor or tuberculosis.

OTTO SAPHIR.

CHEMICAL ORIGIN OF THE HISTOLOGIC SPECIFICITY OF TUBERCULOUS INFLAMMATION. F. ROULET and K. BLOCH, *Virchows Arch. f. path. Anat.* **298**:311, 1936.

When the acid phosphatide obtained by Anderson's method of extraction of the tubercle bacillus is purified, a magnesium salt of the compound is obtained that contains no nitrogen and a constant proportion of phosphorus. This substance when injected intracutaneously leads to the formation of a histologically characteristic tubercle or granuloma, with giant cells. This characteristic tissue reaction is related to the chemical composition of the phosphatid compound, which is taken up in finely dispersed form by the connective tissue cells; these are transformed into epithelioid cells. The phosphatide compound of the tubercle bacillus differs from the phosphatides of other plants in the composition of its saturated fatty acids, to which, when these are liberated by intracellular hydrolysis, the tissue reaction in the last analysis is due. The phosphatide compound obtained from the human bacillus causes a more marked inflammatory reaction in the human skin than in that of the rabbit, and, conversely, the rabbit's skin reacts more strongly to the phosphatide of the bovine bacillus than does that of the human being. This is interpreted as evidence of the chemical specificity of the compound in each of the two strains of bacilli. The reaction is no greater in infected (sensitized) rabbits than in normal ones, which is proof that the inflammatory reaction to specific phosphatide is not the same as the reaction to tuberculin (protein).

O. T. SCHULTZ.

VARIATIONS IN THE CONTENT OF ASCORBIC ACID AND PIGMENT IN THE HEART MUSCLE. K. UHLENBROOCK and R. BOEHMIG, *Virchows Arch. f. path. Anat.* **299**:699, 1937.

Previous work had shown that the lipofuscin content of the cardiac muscle increases with the functional activity of the muscle but that the increase is not uniform throughout the muscle. It had also been found that the vitamin C of the myocardium increases with the functional state of the muscle. In the present work both factors, lipofuscin content and ascorbic acid content, were determined in the same material. Because of its greater mass, the heart of the beef was selected as permitting comparison of different areas of the myocardium. The areas of the left ventricle selected were the subepicardial muscle layer, the sub-endocardial layer, the intervening main muscle mass and the main papillary muscle. A definite difference in content of ascorbic acid was detected, the outer muscle layer being richer in this substance than the rest of the myocardium. Variation in lipofuscin content was also noted; the areas of myocardium poorest in vitamin C were richest in lipofuscin. Vitamin C is a catalytic activator of cellular metabolism. Because of the relation of vitamin to pigment the authors conclude that lipofuscin is identical with melanin.

O. T. SCHULTZ.

EFFECT OF ASCORBIC ACID AND GLUTATHIONE ON EXPERIMENTAL DIPHTHERITIC INTOXICATION. H. LOTZE and S. THADDEA, *Virchows Arch. f. path. Anat.* **300**:685, 1937.

In diphtheria and other severe acute infections and intoxications the adrenal cortex may reveal evidence of damage. The adrenal cortex stores vitamin C. Exhaustion of this vitamin or of other natural reducing substances may explain the failure to obtain beneficial results from the administration of diphtheria antitoxin. In the experiments reported a large number of guinea pigs was used.

A diphtheria toxin (broth filtrate) that would certainly kill the animals in forty-eight hours or less was injected intraperitoneally. Some of the animals were given only the toxin; these served as controls. Others were given simultaneously with the toxin a wide variety of substances, chiefly protein split products. Of the many substances used, none was of any avail except the natural reducing substances of the body, ascorbic acid, cysteine and glutathione. These prolonged the life of the animals in the absence of antitoxin and prevented the changes in the liver, spleen and adrenals noted in controls. Since such changes develop very early in the course of infections and intoxications the reducing substances must be administered early if they are to have beneficial effects.

O. T. SCHULTZ.

Microbiology and Parasitology

EXPERIMENTAL INFECTION OF MICE WITH HAEMOPHILUS PERTUSSIS. W. L. BRADFORD, *Am. J. Path.* **14**:377, 1938.

A characteristic lesion has been produced in the lungs of mice by intratracheal inoculation of suspensions of recently isolated *Haemophilus pertussis*. It consists in interstitial pneumonia with appearance of an excess of mucoid exudate within the smaller bronchi and alveolar spaces. The lesion of the parenchyma is more conspicuous than that of the bronchial epithelium. Pure cultures of *H. pertussis* have been consistently obtained from the infected lungs of mice from ten to twenty days after inoculation. The total leukocyte count has been regularly increased, and extreme degrees of hyperleukocytosis have been observed occasionally.

FROM AUTHOR'S SUMMARY.

ABSCESS PRODUCTION BY FUSIFORM BACILLI IN RABBITS AND MICE BY THE USE OF SCILLAREN B OF MUCIN. R. TUNNICLIFF and C. HAMMOND, *J. Dent. Research* **16**:479, 1937.

A fusiform bacillus isolated three months previously from a patient with ulceromembranous gingivitis produced abscesses about 0.5 cm. in diameter when injected subcutaneously into rabbits and abscesses from 1 to 2 cm. in diameter when injected into skin prepared by injections of 0.1 cc. of scillaren B solution. Although cultures showed only bacillary forms, spirilla-like organisms were demonstrated in smears and sections of the abscesses. Ulceration developed in one scillaren B prepared abscess in which typical spirochetel forms were seen.

This strain of fusiform bacilli nine months after isolation did not produce abscesses in skin or peritonitis in unprepared mice; but abscesses or peritonitis resulted if the mice had previously received scillaren B intradermally or if this drug was mixed with the culture and injected intraperitoneally.

When injections of fusiform bacilli suspended in mucin were made into the skin or peritoneal cavities of mice, abscesses or peritonitis developed, but no lesions resulted from injections of mucin or bacilli alone.

More filaments and fewer spirilla-like organisms were demonstrated in lesions in mice than in lesions in rabbits, although the culture injected was often rough and contained spiral forms.

FROM AUTHORS' CONCLUSIONS.

EFFECT OF TISSUE ENZYME ON PNEUMOCOCCI. R. J. DUBOS and C. M. MACLEOD. *J. Exper. Med.* **67**:791, 1938.

Polymorphonuclear leukocytes contain an enzyme which destroys the basophilic character of heat-killed pneumococci (R and S variants) and inactivates the type-specific polysaccharide antigen of encapsulated cells. The same enzyme, however, fails to cause disintegration of the bacterial cells or to decompose the capsular polysaccharide itself. The enzyme has been extracted from a number of animal tissues. It appears identical with a purified enzyme extracted from pan-

creatin, and it decomposes yeast nucleic acid. These facts are considered with regard to the failure of rabbits to produce antibodies for the type-specific carbohydrate when immunized with heat-killed encapsulated pneumococci by the intradermal route.

FROM AUTHORS' SUMMARY.

CORYZA OF DOMESTIC FOWL. J. B. NELSON, J. Exper. Med. **67**:847, 1938.

Coccobacilliform bodies were regularly demonstrable in addition to *Haemophilus gallinarum* in exudate from birds infected with a passage strain of a coryza of rapid onset and long duration (type III). Both agents were present throughout the entire course of the disease. The characteristics of type III coryza were reproduced by injecting a mixture of the two agents. The behavior of each component was altered by the association, indicative of a synergistic relation. Evidence that the coccobacilliform bodies might occasionally develop in birds infected with *H. gallinarum* following transfer by indirect contact was also obtained. The combined action of the two infective agents adequately accounts for the development of this particular strain of type III coryza.

FROM AUTHOR'S SUMMARY.

CULTIVATION OF VACCINIA VIRUS WITH RETICULO-ENDOTHELIAL CELLS. J. W. BEARD and P. ROUS, J. Exper. Med. **67**:883, 1938.

The pathogenic activity of vaccinia virus is in large part suppressed when the virus is mixed with living Kupffer cells or clasmotocytes in the test tube and injected intradermally. This virus increases in quantity when introduced into such cultures in vitro and survives in immediate association with these cells. No anti-viral principle is elaborated by the Kupffer cells under such conditions.

FROM AUTHORS' SUMMARY.

ORGANISMS RECOVERED FROM FILTRATES OF CULTURES OF HEMOLYTIC STREPTOCOCCI. E. E. NICHOLLS, J. Infect. Dis. **62**:300, 1938.

The results of the present study tend to confirm further the theory that when streptococci are aged under unfavorable conditions small viable forms are produced which are capable of passing through filters and which may assume visible forms when submitted to suitable conditions. Whether these filter-passing forms represent a filtrable stage of the life cycle or are merely viable particles that had occasionally escaped through the filter cannot be determined by the results obtained in this study. However, the influence of the porosity of the filter in the recovery of the organisms tends to favor the latter view.

FROM AUTHOR'S SUMMARY.

TYPE-SPECIFIC BACTERIOPHAGES FOR CORYNEBACTERIUM DIPHTHERIAE. E. V. KEOGH, R. T. SIMMONS and G. ANDERSON, J. Path. & Bact. **46**:565, 1938.

The condition produced in the skin of the rabbit by the injection of live pneumococci is inhibited by the addition of type-specific antipneumococcus serum in sufficient quantity to the dose of bacteria injected. Antipneumococcus serum inhibits the growth of the bacteria when injected with them in sufficient quantity. A method is described which permits comparison of the effects of varying doses of two antipneumococcus serums on a constant dose of live culture in the skin of the same rabbit. The potency in international units obtained by this method approaches that found by the mouse protection test. The accuracy of comparing two serums intracutaneously in one rabbit is equal to that of the mouse protection test when injections are made into 200 mice. The nature of the effect of antipneumococcus serum observed by the rabbit intracutaneous method is discussed. The two reactions described may be caused by qualitatively different properties of the serum.

FROM AUTHORS' SUMMARY.

EARLY CHANGES IN THE INTIMA OF THE INTRAHEPATIC BRANCHES OF THE PORTAL VEIN IN INOCULATION TUBERCULOSIS OF THE GUINEA PIG. F. HAUSBRANDT, *Virchows Arch. f. path. Anat.* **301**:223, 1938.

From five to ten weeks after subcutaneous inoculation of various strains of tubercle bacilli in guinea pigs the livers were removed, and the intima of branches of the portal vein were studied for early changes. The earliest reaction was exudative, consisting in swelling and partial desquamation of the endothelium. This was followed by swelling of the subendothelial tissues and proliferation of large subendothelial mononuclear cells. These large cells were transformed into epithelioid cells, and finally the characteristic tuberculous lesion resulted and fused with the perivascular lesion. Hausbrandt's concept of the course of events is that the bacilli penetrated the portal precapillary vessels without detectable change in the intima or media. Then a characteristic tuberculous perivascular lesion developed. The intimal changes observed developed secondary to the perivascular lesion and were not the result of localization of blood-borne tubercle bacilli directly in the intima.

O. T. SCHULTZ.

TETANUS BACILLI IN THE CARDIAC BLOOD AND SPLEEN. W. PIRINGER, *Zentralbl. f. Bakt. (Abt. 1)* **141**:375, 1938.

Piringer reports a case of acute tetanus in which the tetanus bacillus was recovered on two occasions from the wound before death and from the cardiac blood and spleen after death. He emphasizes the importance of more frequent efforts to cultivate tetanus bacilli from the blood in wound infections and the probable importance of serum therapy in preventing tetanus-bacillemia.

PAUL R. CANNON.

Immunology

HUMAN INFLUENZA IN SWINE. R. E. SHOPE, *J. Exper. Med.* **67**:739, 1938.

Antibodies capable of neutralizing human influenza virus were present in the serum of old swine on two New Jersey institutional farms but absent from the serum of young swine on the same farms. The old animals had lived through the winter of 1936-1937, in which outbreaks of disease of the upper part of the respiratory tract were prevalent among the human inmates of the two institutions, while the young swine studied were born long after these outbreaks. It is believed that the swine the serum of which neutralized human influenza virus had undergone an unrecognized infection with human influenza virus, acquired from man. The possible bearing of these observations on the theory that swine influenza was originally of human origin is discussed.

FROM AUTHOR'S SUMMARY.

IMMUNIZATION WITH ANTIGEN FROM PNEUMOCOCCI. R. J. DUBOS, *J. Exper. Med.* **67**:799, 1938.

Pneumococci killed by acetic acid at p_H 4.2 and then allowed to become gram-negative at p_H 7 under conditions such that no cellular disintegration takes place release in solution small amounts of a substance which is precipitable by acetic acid and soluble at a neutral reaction. This soluble fraction injected into rabbits by the intravenous route causes production of antibodies which afford definite protection to mice infected with virulent pneumococci of types I, II and III. Other types were not tried. White mice immunized with this soluble antigen exhibit some active immunity to virulent pneumococci, but the results have been very irregular so far. Soluble fractions, similar in properties and with apparently the same immunizing action, have been obtained from both virulent (S) and avirulent (R) pneumococci.

FROM AUTHOR'S SUMMARY.

QUANTITATIVE PROTECTION IN MONKEY MALARIA. L. T. COGGESHALL and M. D. EATON, *J. Exper. Med.* **68**:29, 1938.

The minimal infective dose of *Plasmodium knowlesi* for rhesus monkeys was found to be between 1 and 10 parasites when injected intraperitoneally. As the dose of parasites is increased, the length of time prior to the appearance of circulating parasites is decreased. However, the severity of the infection, once it is established, is independent of the initial dose of parasites. In experimental passive protection a quantitative relationship was demonstrated between the number of parasites in the inoculum and the effective amount of immune serum given at the time of infection and in equal doses daily for nine days thereafter: The smaller the inoculum the less was the quantity of immune serum required to prevent the death of the animal. In experimental protection, when relatively large amounts of immune serums and small numbers of parasites were used, infection was prevented.

FROM AUTHORS' SUMMARY.

BACTERICIDAL ACTION OF PROSTATIC FLUID IN DOGS. G. P. YOUNG, J. LIEBLING and R. Y. LYMAN, *J. Infect. Dis.* **63**:117, 1938.

Prostatic fluid was collected aseptically from normal dogs and tested for germicidal action on colon bacilli, staphylococci (*Staph. aureus*), streptococci and gonococci. Marked germicidal power was demonstrated for the colon bacilli, the staphylococci and the streptococci and very slight germicidal power for the Torrey strain of the gonococci. Different samples of prostatic fluid varied considerably in germicidal action. The samples were tested for complement and lysozyme, and neither of these factors could be demonstrated. Adsorption with either *B. coli* or *Staph. aureus* removed the germicidal agent, which was apparently nonspecific. Heating at 50 C. for thirty minutes slightly reduced the germicidal power; heating at 55 C. reduced the germicidal power over 50 per cent, and heating at 60 C. for thirty minutes completely destroyed it. The prostatic fluid could be kept in the ice box for over three months without loss of potency. As tested, the germicidal power of prostatic fluid from a dog was far greater than that of blood serum taken from the same animal.

The results give no indication of the nature of the germicidal substance present in normal prostatic fluid, but the active factor does not appear to be similar to the factors that have been found to be responsible for germicidal effects in other body fluids and excretions.

FROM AUTHORS' SUMMARY.

SEROLOGIC PROPERTIES OF A POLYSACCHARIDE FROM THE MUCOID SUBSTANCE OF HEMOLYTIC STREPTOCOCCI. H. LOEWENTHAL, *Brit. J. Exper. Path.* **19**:164, 1938.

From hemolytic streptococci in the mucoid phase and also from type III pneumococci a substance can be isolated which has nature of a polysaccharide. Its serologic activity can be demonstrated only with serum produced by the use of cautiously killed suspensions of young encapsulated cocci. It is neither type specific nor species specific, and it appears to be unconcerned with the invasive properties of streptococci. The fact that streptococci in the mucoid phase stimulate the formation of protective antibodies and precipitins for the polysaccharide from mucoid material only when in the young encapsulated state can be explained by the process of deterioration of the bacterial surface which sets in after several hours of growth.

FROM AUTHOR'S SUMMARY.

INCREASE OF THE ANTIGENIC PROPERTY OF SERUM BY ULTRAVIOLET IRRADIATION. W. STECHER, *Virchows Arch. f. path. Anat.* **300**:645, 1937.

The literature contains conflicting reports on the effect of ultraviolet irradiation on the antigenic property of serum. Some ascribe to this procedure an

increase in allergic activity; others claim that the serum is denatured and loses its antigenic property. In the author's experiments, when rabbits were sensitized with untreated swine serum and then given irradiated serum as the provocative dose no anaphylactic response was elicited. However, when the animals were sensitized with the irradiated serum and given the same serum in the provocative injection 3 animals died in anaphylactic shock. The animals that survived revealed marked hyperergic inflammatory reactions. The author's findings are based on a small number of animals, 5 in each of the two experiments. O. T. SCHULTZ.

PREPARATION OF TYPHOID VACCINE WITH MERCURIC CHLORIDE. D. BARTOS and J. BUCHGRABER, *Zentralbl. f. Bakt. (Abt. 1)* **141**:355, 1938.

A 4 per cent solution of mercury bichloride kills typhoid and paratyphoid bacilli more rapidly and produces a vaccine less toxic than the one prepared by the use of heat. Agglutinin produced by such a vaccine appeared earlier and in higher titer than that obtained by injecting comparable amounts of heat-killed vaccine. When injected into human subjects, the mercury bichloride vaccine caused a less severe reaction both locally and generally than did the heated vaccine.

PAUL R. CANNON.

QUANTITATIVE DETERMINATIONS OF THE FRACTIONS OF COMPLEMENT. A. HEGEDUES and H. GREINER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **92**:1, 1938.

Practical methods for the separation of the four fractions of complement are presented. The titer of complement depends on the action of that fraction of which there is the least amount. The fractions cannot replace each other; i. e., an abundance of one will not compensate for the deficiency of another. The quantities of the fractions differ in different species. The serum of the guinea pig has the highest complementary action, then follow in the order of their respective titers the serums of: rat, man, hog, dog and rabbit. Man has a great deal of the thermostable fourth fraction (inactivated by ammonium hydroxide) and very little of the thermolabile albumin fraction, of which a great deal is present in the serum of the guinea pig. The thermostable third fraction (inactivated by cobra venom and by yeast) is particularly abundant in the serum of the hog and the thermolabile globulin fraction in the serums of cattle, horse, sheep and squirrel.

The serums of the four last-mentioned species showed a failure of complementary action, which was due to deficiency in (only traces) or complete absence of all the fractions except the globulin fraction.

I. DAVIDSOHN.

USE OF IMMUNE SERUM IN THE TREATMENT OF ANTHRAX IN BULGARIA. P. KOSCHUCHAROFF, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **92**:53, 1938.

Among a population of about 6,000,000, there occur from 800 to 1,100 cases of anthrax. From 2,000 to 3,000 cases occur in domestic animals. The infection is contracted almost without exception by contact with diseased animals. The report is based on 521 cases in man. In each case the malignant pustule was very characteristic, and bacteriologic studies were superfluous. In most cases it was located on the exposed parts (face, arms, legs), but no part of the body was free. The mortality was greatest among those who had the primary lesion on the face. After a historical review of surgical and chemical therapy, the author presents the great successes of serum therapy. The administration of large doses led to improvement in results, the mortality dropping from 33 per cent in 1912, with average doses of from 30 to 150 cc., to 3 per cent in 1935 and 1936, with larger average doses, reaching in some cases 1,500 cc. The methods of producing

immune serum are reviewed. It is emphasized that no satisfactory method of standardization of anthrax immune serum is available and that normal serum is often just as efficacious as immune serum.

I. DAVIDSOHN.

BLOOD GROUPS IN NOMADS OF ASIA MINOR. S. IRMAK, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **92**:74, 1938.

In Anatolia there are about 500,000 nomads of pure Turkish racial stock known as Yürük. They do not intermarry with the sessile population. A determination of the blood groups among 400 of them revealed the following distribution: O, 51.3 per cent; A, 40.5 per cent; B, 6.2 per cent, and AB, 2 per cent. The biochemical index was 5.2 per cent. This distribution is strikingly different from the old finding of Hirszfeld. The discrepancy is explained by the fact that Hirszfeld studied Turkish soldiers of different racial stocks. From his observations, Irmak draws far reaching conclusions concerning the validity of Bernstein's idea of the origin of the factor B and concerning the anthropologic relations between the Turks and the so-called Alpine races.

I. DAVIDSOHN.

Tumors

PROGNOSTIC SIGNIFICANCE OF INTRACELLULAR MUCICARMINOPHILIC MATERIAL IN CARCINOMA OF THE FEMALE BREAST. V. K. FRANTZ, *Am. J. Cancer* **33**:167, 1938.

One hundred and thirty cases of operable carcinoma of the female breast have been analyzed after radical mastectomy according to the presence or absence of axillary metastases, the presence or absence of intracellular mucicarminophilic material and the grade of tumor, according to the plan submitted by Haagensen. The follow-up on the series includes 97.1 per cent for a five to nine year period. The mucicarminophilic material is found in all grades, but the number of cases in which it is found decreases with increase in histologic evidence of malignancy. The relative amount of the material is found to be related to the length of life after operation. The evidence presented indicates that the presence or absence of mucicarminophilic material provides additional information for estimating the malignancy of tumors of grade 2. It is pointed out that no histologic character yet found carries nearly the weight in prognosis that is carried by well known clinical findings, such as age, extent of local disease and presence of metastases. Moreover, there are probably many other factors of which there is little knowledge at present which determine the outcome.

FROM AUTHOR'S SUMMARY.

MALIGNANT TUMORS IN DOGS. H. B. RUGGUCK and R. A. WILLIS, *Am. J. Cancer* **33**:205, 1938.

Nine selected malignant tumors in dogs are described, and their characters compared with the corresponding tumors of man. The tumors described comprised carcinoma of the tonsil 2 cases, carcinoma of the cardia of the stomach, carcinoma of the pancreas, carcinoma of the prostate, carcinoma of sweat glands, cystic and papillary carcinoma of the breast, mixed adenocarcinomatous and osteosarcomatous tumor of the thyroid, and primary sarcoma of the heart.

FROM AUTHORS' SUMMARY.

CANCER OF THE ALIMENTARY TRACT IN MICE. H. G. WELLS, M. SLYE and H. F. HOLMES, *Am. J. Cancer* **33**:223, 1938.

This article is an extension of a similar report made twenty years ago. It emphasizes again the striking infrequency of cancer in the alimentary tracts of animals of all species other than man, whose digestive canal is the site of approxi-

mately half of all carcinoma occurring in man. In 142,000 mice of the Slye stock, all dying of natural causes, without experimental manipulation, most of them of cancer age, only 15 primary malignant neoplasms of the stomach have been found, 8 being squamous cell carcinoma of the cardia, 3 adenocarcinoma of the pylorus, 3 apparently a benign epithelial growth, and 1 primary sarcoma. In the literature the authors could find reports of but 7 other cases of gastric cancer in mice. There were 19 primary intestinal neoplasms, of which 11 had arisen in prolapsed rectums: 6 were squamous cell carcinoma, 2 adenocarcinoma and 3 sarcoma in mice of a sarcoma strain, and only 1 was adenocarcinoma. There was also 1 instance of squamous cell carcinoma of the anus. In the literature only 6 other cases of cancer in the intestines of mice could be found recorded. Not a single neoplasm of the esophagus was found in these 142,000 mice, which had produced many thousands of other tumors, and the authors could find in the literature no record of a cancer of the esophagus in mice. The literature of cancer of the alimentary canal in animals is reviewed briefly, and the incidence contrasted with that in man.

FROM AUTHORS' SUMMARY.

ACTION OF X-RAYS ON EGG FRAGMENTS. P. S. HENSHAW, *Am. J. Cancer* **33**:258, 1938.

Experiments have been carried out to determine whether a certain radiobiologic effect in cells results from changes produced in the nucleus or in some other part of the irradiated cells. The effect referred to was a delay in cell division. The test material used was eggs of *Arbacia punctulata*. Nucleated and non-nucleated egg fragments were obtained by the centrifuge method, both of which will undergo fertilization and development. These were irradiated and inseminated, and the moment when 50 per cent had divided was determined. It was found that for a given dose of radiation an appreciable delay was produced in whole eggs and nucleated fragments of about the same extent in each, whereas no delay was produced in the non-nucleated fragments. Thus, since there is direct correlation between the presence of a nucleus and the manifestation of the irradiation effect and since changes occurring in parts exclusive of the nucleus produce no observable effect, it appears that the slowing in cell division brought about in the eggs of *Arbacia punctulata* by roentgen rays is due to changes produced directly in the nucleus by the radiation.

FROM AUTHOR'S SUMMARY.

A CLINICOPATHOLOGIC STUDY OF ASTROCYTOMA. R. W. WAGGONER and K. LÖWENBERG, *Arch. Neurol. & Psychiat* **38**:1208, 1937.

Waggoner and Löwenberg state that astrocytoma involves the hemispheres as well as the brain stem and very often grows bilaterally, in contrast to other types of glioma, the growth of which is usually unilateral. Sections of the whole brain harboring astrocytoma were stained by the method of Weigert, and the areas of demyelination were traced. On the location of the nucleus of the tumor depends the further mode of invasion. For instance, when the nucleus of the tumor is in the corpus callosum, the growth is bilateral and invades the frontal lobes and the anterior parts of the parietal lobes. If the nucleus of the tumor is in the splenium of the corpus callosum, the growth invades the temporal and occipital lobes and both basal ganglions. Unilateral astrocytoma seldom invades the corpus callosum; it preferably invades the pyramidal tract.

GEORGE B. HASSIN.

THE FAMILIAL ASPECTS OF CANCER. H. L. LOMBARD, *New England J. Med.* **218**:711, 1938.

All the methods of study used point toward some hereditary tendency to cancer. They do not indicate whether all cancer is hereditary. The statistical means at one's disposal make it impossible to investigate this point. A general hereditary

susceptibility may or may not be present. This study indicates merely that persons among whose immediate relatives cancer has occurred have a slightly greater chance of contracting the disease than has the remainder of the population. The difference between the two groups is not sufficient to cause undue worry, but indicates without any question that further statistical studies on this problem are desirable.

FROM AUTHOR'S SUMMARY.

THE CHEMICAL COMPOSITION OF THE ACTIVE AGENT OF THE ROUS SARCOMA
No. 1. A. POLLARD, Brit. J. Exper. Path. **19**:124, 1938.

The tumor-producing agent of Rous sarcoma 1 can be deposited from cell-free extracts by means of the Sharples centrifuge. The resuspended deposit can be purified further by fractional centrifugation. The product obtained consists mainly of protein, with a variable amount of lipid material. Preparations of lipid-extracted tumor desiccates contain much smaller amounts of lipid but retain their tumor-producing activity.

FROM AUTHOR'S SUMMARY.

SPECIFIC COMPLEMENT-FIXATION WITH THE VIRUS OF SHOPE'S FIBROMA. C. E.
VAN ROOYEN, Brit. J. Exper. Path. **19**:156, 1938.

Rabbits immunized with either the IA or OA strain of the virus of Shope's fibroma in the living state had complement-fixing as well as virus-neutralizing properties in their serums. The complement fixation reactions afforded no basis of distinguishing between the IA and OA strains. Complement-fixing antibodies appeared about two to three weeks after injection of the virus. They usually disappeared about eight weeks after the last injection of virus. Virus heated to 55 C. for one hour retained its antigenic properties, giving rise to complement-fixing and neutralizing antibodies. Notwithstanding repeated doses of living (or dead) virus, no complement-fixing antibodies appeared in the serum of certain rabbits. The serum of such animals, however, showed a powerful virus-neutralizing effect. Therefore, though all serums yielding complement fixation also neutralized the virus, it could not be said that all serums showing neutralization of the virus showed the presence of complement-fixing antibodies.

FROM AUTHOR'S SUMMARY.

GIANT CELL TUMOR OF BONE. E. K. DAWSON, J. R. M. INNESS and W. F.
HARVEY, Edinburgh M. J. **45**:491, 1938.

Giant cell tumor of bone is a neoplastic growth, probably originating with trauma and hemorrhage and developing as an osteogenic reaction and proliferation, which may slowly progress and ultimately regress, but which in rare cases acquires the characters of osteogenic sarcoma. It may be classed as a type of new growth intermediate between purely reactive tissue and true blastoma.

FROM AUTHORS' SUMMARY.

MICROMANIPULATION AND MICRODISSECTION OF THE INCLUSION BODY OF MOL-
LUSCUM CONTAGIOSUM. C. E. VAN ROYEN, J. Path. & Bact. **46**:425, 1938.

Fresh material obtained in 10 cases of human molluscum contagiosum has been stained *intra vitam* with brilliant cresyl blue and subjected to microdissection. The inclusion body of molluscum contagiosum has been successfully removed from the infected epithelial cell. It has also been replaced in the same cell without injuring the latter. The removal of the inclusion body leaves a large cavity in the cell. The cytoplasm does not flow into this space, the cavity being apparently lined by a localized condensed layer of cytoplasm. The boundaries of this cavity have been defined by injecting india ink into it or alternatively by introducing india ink into the surrounding cytoplasm. The inclusion body prob-

ably grows from a more minute form of the virus. It is not formed by desiccation or by fusion of any intracytoplasmic constituents of the cell. It is suggested that the large oval or pear-shaped form of the inclusion body represents a later stage of development than the smaller spherical one. The large pear-shaped inclusion bodies have been shown to possess a definite outer membrane, which is thinnest at the conical pole. These observations have been established by demonstrating the tensile strength of the membrane on "palpation" with the point of a microneedle. The weakness of the conical segment has also been revealed by transfixing the inclusion body between two needles and exerting traction on its walls. The elementary virus bodies contained within the inclusion body of *molluscum contagiosum* have been shown to be supported in a gelatinous mucoid substance.

FROM AUTHOR'S SUMMARY.

IMMUNITY AGAINST CARCINOMA IN THE RABBIT. A. BESREDKA and L. GROSS. *Ann. Inst. Pasteur* 60:5, 1938.

Brown-Pearce carcinoma was usually fatal when injected into the testicle, brain or anterior chamber of the eye of the rabbit. The mortality after subcutaneous injection was lower. Intracutaneous inoculation produced a benign tumor, which was ultimately resorbed and which rendered the animal immune. Rabbits naturally refractive have never been found.

The carcinomatous material was attenuated so that it did not produce tumors by storage at 0 C. for twenty-one days or at 37 C. for from two to three days, by addition of bile or glycerin or by freezing and thawing. Rabbits treated with attenuated material were not immune to active carcinoma.

Intracutaneous inoculation of unattenuated material uniformly resulted in solid and lasting immunity. Tumors could not be produced in immune rabbits regardless of the size of the dose or of the route of administration. This immunity was entirely cellular and could not be transmitted passively. Serum from immune animals in doses of 50 cc. had no specific effect whether given before or after the introduction of the epithelioma. Experiments in parabiosis also failed to show any evidence of passive transfer.

J. B. GUNNISON.

BASILAR AND SACRAL CHORDOMA. H. VON BRAITENBERG, Frankfurt. *Ztschr. f. Path.* 50:509, 1937.

The author reviews the recent literature and cites the classification of chordoma according to Coenen and Linck. Five tumors of this type are then presented. Three of these were basilar chordoma. Two were benign. The third was malignant and composed chiefly of the large vesicular cells characteristic of chordoma, with intracellular spaces containing much mucoid material. Two of the tumors were malignant sacral chordoma with invasion of the surrounding bones. The author believes that chordoma belongs to the dysontogenetic types of tumor, and that it arises from remaining chorda complexes in the perivertebral tissue. In chordoma are found cells typical of all the different stages of development of the chorda dorsalis, the early cell types being present chiefly in the malignant forms. The amount of mucoid material is larger in malignant than in benign chordoma. This material is thought to be the product of degenerated cells. Also, glycogen, which is present in the cells of the chorda dorsalis, is found in the tumor cells.

ANNEMARIE STRAUSS.

CULTIVATION OF ADULT TISSUES IN HUMAN SERUM. R. TASZKAN, *Virchows Arch. f. path. Anat.* 299:106, 1937.

It was found possible to cultivate adult human and animal tissues in human serum in the absence of chick embryo extract. Chicken plasma and human serum in equal quantities of 0.5 cm. were used, and the tissue fragment was implanted.

After coagulation of the medium, 1 cc. of Ringer's solution was added. After two days of incubation, the liquid was pipetted off and replaced by human serum diluted 1:4 with Ringer's solution. Thereafter the liquid portion was changed every five days. By this procedure it was possible to cultivate connective tissue of the spleen, heart, kidney, placenta and liver and epithelium of the kidney and liver. The growth-promoting factor is held to be derived from cells in which the membrane has been injured and into which unassimilable substances have penetrated. The injury to the cells is overcome by growth. The serum of pregnant women and of persons with malignant tumors supported growth better than did normal human serum. The disintegration of tissue within the organism leads to increased proteolysis; the proteolysins are active only against the denatured proteins of the organism from which they are derived.

O. T. SCHULTZ.

THE SO-CALLED ASTROCYTOMA OF THE CEREBELLUM. H. BERGSTRAND, *Virchows Arch. f. path. Anat.* **299:725**, 1937.

Cushing noted that astrocytoma of the cerebellum differs from cerebral astrocytoma in the fact that it occurs predominantly in children and young adults and in the fact that a much better prognosis may be given if the tumor is removed surgically. In 1932 Bergstrand reported the results of a study of 11 examples of this tumor and concluded that the term "astrocytoma" is a misnomer. He now reports on 45 additional tumors of this group. Of the 56 tumors, 18 occurred in the first decade of life, 15 in the second and 16 in the third. So-called astrocytoma may involve the pia as well as the parenchyma of the cerebellum, in which case the pia contains heterotopic tissue. The cerebellar tissue adjacent to the tumor reveals malformation. The stratum granulosum is reduced, Purkinje cells are absent, and on the surface of the molecular layer is an added zone of neurofibrils and glia by means of which the tumor is attached to the pia. The tumor itself represents congenital maldevelopment. "Astrocytoma" is an improper name for it because the growth consists of embryonic glia cells, neuroblasts, axis-cylinders and medullated nerve fibers. It should be termed glioneuroblastoma. Areas of the tissue may become differentiated, fibrous and very hard. Because the tumor is congenital, few of its victims reach the age of 25 years unless the tumor has been surgically removed.

O. T. SCHULTZ.

EPITHELIAL CHANGES IN THE FEMALE BREAST IN RELATION TO AGE AND CANCER. B. KÖRPÁSSY, *Virchows Arch. f. path. Anat.* **299:793**, 1937.

Presumably normal breasts obtained at necropsy from 300 women aged 13 to 89 years were examined histologically with special reference to epithelial proliferation. In each instance both breasts were examined. Dilatation of small ducts into cysts was so common as to be almost physiologic, being observed in two thirds of the cases. Duct dilatation with epithelial proliferation occurred in 48.7 per cent of all the cases, and the incidence as well as the extent increased progressively with age, the incidence reaching a maximum of 75.5 per cent in women over 70 years of age. The proliferated epithelium was of the small cuboidal type that normally lines the ducts or of the tall pale apocrine type. Although the degree and frequency of epithelial proliferation increased with age, no changes suggestive of invasion or early malignant growth were observed. In a woman aged 80 years histologic examination revealed a small carcinoma in each breast; neither growth was associated with other epithelial proliferation. In a woman aged 64 a pea-sized carcinoma of the breast was discovered; in the involved breast there was proliferation of duct epithelium. Statistical analysis of the author's material in comparison with a series of carcinomatous breasts established that in cancerous and noncancerous breasts there is a progressive increase in degree and frequency of epithelial proliferation with advancing years and that epithelial proliferation is more frequent in breasts with cancer than in those without. Although

the author's material yielded no evidence of direct transformation of the epithelial proliferation of cystic disease of the breast to carcinoma, he looks on the epithelial proliferation as the last stage before cancer.

O. T. SCHULTZ.

THE ASSOCIATION OF PACHYMEINGITIS WITH TUMORS. E. LASS, Zentralbl. f. allg. Path. u. path. Anat. 69:404, 1938.

In a series of 46 bodies with pachymeningitis haemorrhagica interna there were 6 which had cellular nodules attached to the dura. These nodules seemed to arise from the inner aspect of the dura. They protruded above the surface of the thin young membranes and were buried deeply in the thick old membranes. The cells of the protrusions resembled those coating the dura, and most clumps contained numerous psammoma bodies. There seemed to be no relation between the occurrence of such nodules and the age of the patient or the thickness or extent of the pachymeningitis.

The richest nodular hyperplasia was encountered in the body of a woman 85 years old who had marked arteriosclerosis. Her brain was altered by softening. The dura of the right middle and posterior cranial fossae was covered by pachymeningeal membrane and contained linseed-sized firm nodules. As a differential diagnostic aid, Lass cites the case of a woman whose dura was altered extensively by metastases from a cancer of the breast. The nodules encountered were veins filled with tumor cells.

The possibility is advanced that nodular hyperplasia is related to true neoplastic growth, such as meningioma. Hyperplasia occurs also in sites unassociated with pachymeningeal membranes and possibly is related to some vascular disturbance. It is known that new capillary formation occurs on the inside of the dura when the veins on the outside are obstructed. New vessel formation does not occur when the veins on the inside of the dura are occluded.

GEORGE J. RUKSTINAT.

Society Transactions

PATHOLOGICAL SOCIETY OF PHILADELPHIA

BAXTER L. CRAWFORD, *President*

Regular Meeting, May 12, 1938

HERBERT L. RATCLIFFE, *Secretary*

OSSEOUS CHANGES IN MAN FOLLOWING PROLONGED INGESTION OF FLUORIDES: REPORT OF A CASE. JOHN T. BAUER.

Chronic fluoride poisoning in man has been manifest in two forms: (1) mottled enamel, or dental fluorosis and (2) osteosclerosis. An instance of the latter occurred in a Negro laborer who for eighteen years had handled finely ground rock phosphate, a sample of which contained 3.88 per cent fluorine. He was admitted to the medical service of Dr. R. H. Farley at the Pennsylvania Hospital because of syphilitic aortitis. Roentgenologically the osseous changes were increased density, loss of detail despite increased exposure and lack of normal sharpness along the tendinous attachments, with formation of osteophytes and exostoses. These changes were greatest in the vertebrae, ribs and sternum and least in the long bones of the extremities. Gross examination of the bones confirmed the roentgenologic report of osteophytes, exostoses and roughening of the muscular attachments. Also noted were: (a) chalky white patches on the surfaces, (b) plaques of thin cortical bone and (c) increased thickness of the cortex of the ribs and, to a less extent, of the bones of the extremities. Microscopically, the cortex of the ribs encroached on the marrow spaces, but amorphous granules were not observed. Determinations of the specific gravity of the bones indicated that they were less dense than normal; hence the increased opacity to irradiation was thought to be due to increased formation of bone. Chemically, the bones had from ten to twenty times the normal fluorine content. The bones with the greatest amounts had given the greatest differences roentgenologically in life. Analyses for ash, calcium, phosphorus and carbon dioxide gave normal values.

Clinically and pathologically the patient did not have anemia or other changes which could be ascribed to the prolonged ingestion of fluorides. From the standpoints of the roentgenologist (Bishop, P. A.: *Am. J. Roentgenol.* **35**:577, 1936), the pathologist (Bauer, J. T.; Bishop, P. A., and Wolff, W. A.: *Bull. Ayer Clin. Lab., Pennsylvania Hosp.* **3**:67, 1937) and the chemist (Wolff, W. A., and Kerr, E. G.: *Am. J. M. Sc.* **195**:493, 1938), the observations on this case indicate that it agrees in general with the two others similarly studied and reviewed by Roholm (Fluorine Intoxication, London, H. K. Lewis & Co., Ltd., 1937).

NOTES ON A FORM OF RENAL DISEASE OF THE DOG. H. M. MARTIN.

Of domestic animals, the dog seems to be the most commonly and most seriously affected by renal disease. And perhaps the most frequent and important lesion of the kidney of the dog is a kind of interstitial nephritis which often appears as a sequel to distemper, especially when this condition is complicated by pneumonia. Other acute infections also may be considered as causative factors.

The acute stages of interstitial nephritis are not often seen at necropsies, probably because the animals usually survive these stages and the disease progresses to the subacute or chronic phases with scar formation. Thus the "inflammatory shrunken kidney" of the dog is believed to be identical in most instances with the late stages of interstitial nephritis.

In the acute phases of this disease the capsular surface of the kidney is elevated by well circumscribed grayish or yellow-gray foci of variable dimensions. These are more or less closely placed over the organ. On the cut surface similar infiltrations occur as small circumscribed nodules or wide irregular areas obscuring the usual markings and causing widening of the cortex so that it comes to approximate the width of the medulla.

The histologic changes vary with the gross appearances, but in all a constant feature is more or less extensive infiltration of the interstitial tissues by cells of the lymphocyte series, chiefly large forms, with small numbers of polymorphonuclear leukocytes. These cells accumulate principally in the cortex, but in severe cases the medulla is involved also. Occasionally the medulla alone is infiltrated. Small blood vessels also contain increased numbers of lymphocytes. The tubules and glomeruli are essentially unchanged at this time.

These acute phases of the disease apparently are followed by scarring with the result that the kidney may come to have an appearance resembling that which follows multiple infarction or pyelonephritis; or scar formation may be diffuse, and a small granular, tough, leathery organ result. With these changes the cortex is narrowed greatly.

The microscopic appearance of such organs may be similar in certain respects to that due to chronic glomerulonephritis, and differentiation may be difficult. However, glomerular changes in this instance appear to be part of the general interstitial disease and affect first Bowman's capsule with secondary disease of the capillary tuft. Calcification of the glomerular capsule with fibrosis and calcification of the tuft may occur, or glomeruli may appear hypertrophic. An interstitial infiltrate of lymphocytes may be seen about the scarred areas, suggesting progressive disease.

The clinical signs of interstitial lymphocytic nephritis vary. Often the first evidence of disease is ulcerative stomatitis. There may be also marked visual disturbances or ascites or hydrothorax. Usually anemia is present. Probably the most common terminal change is severe hemorrhagic gastroenteritis.

That renal function is impaired may be shown by an analysis of the blood. The specific gravity of the urine is low, albumin is present in variable amounts, and casts, leukocytes and erythrocytes are often found.

LEUKOSIS OF FOWLS. E. L. STUBBS.

"Leukosis" is a term used to include the leukemia as well as the leukemia-like diseases of fowls. Several different types of leukosis have been described, some of which are transmissible (a fact which facilitates study of these types) while others cannot be transmitted and are not well understood.

Transmissible types are readily transferred from diseased to normal chickens by injections of blood, organ emulsions or filtrates. The causative agent is more active in the blood cells than in the plasma and filtrates. Transmission succeeds best with injection of whole blood. In tests made thus far the causative agent has resisted drying from the frozen state for at least nine hundred and thirty-two days.

The period of incubation varies from one week to four months. The younger the chickens the more susceptible they are as measured by the period of incubation and the mortality. Natural methods of spread are not well understood. Mosquitoes and mites have been tried as vectors of this disease, without success.

There have been reported strains of leukosis that produce leukemia when injected intravenously and tumors when injected locally. Apparently such strains stimulate cells of more than one type to unrestricted growth. Mixed strains may produce a wide variety of changes. Some strains appear pure and produce results of only one type: the stimulation of the hemopoietic tissues to abnormal activity.

TUMORS OF THE NERVE SHEATHS IN FISH. BALDUIN LUCKÉ.

The tumors reported here closely resemble the complex group of human neoplasms arising from nerves. They were found in 39 fish belonging to three

species of snappers. Usually but one growth was present, though occasionally several were noted. The tumors were firm and resilient and usually had a white cut surface. Histologically they showed considerable variation in structure. They usually had a distinctly fasciculated makeup, and the cells were arranged in interlacing bands or whorls. In many tumors the oval nuclei were grouped in more or less parallel rows or palisades, between which were dense masses of fibrils.

The relative ease with which these tumors may be secured in tropical waters renders them favorable material for the study of an important group of neoplasms.

NEOPLASTIC DISEASE OF THE PANCREAS IN REPTILES. HERBERT L. RATCLIFFE.

Some time ago, in the course of autopsies at the Zoological Garden, enlargement of the pancreas of a pine snake was noted. In sections of this organ much of the normal tissue seemed to have been replaced by neoplastic epithelium (Ratcliffe, H. L.: *Am. J. Cancer* 24:78, 1935). As a result of this observation histologic study of the pancreas in reptiles became a routine practice, and apparent tumor formation has proved to be relatively common.

Thus far the disease has been limited to snakes, the greater number of which have represented North American species. In 72 pancreases from these animals the gross appearances have not been informative, but sections revealed in 15 extensive destruction of the parenchyma by tissue that resembled carcinoma. Focal changes that suggested early stages of tumor growth were found in 9 other specimens.

According to my present interpretations, the earliest stages of the disease are small areas within the organ that are occupied by abnormal branching ducts, loosely arranged and pale-staining, among which are fragments of glandular tissue. These ducts are composed of irregular, indistinctly outlined flattened epithelial cells, the nuclei of which are variable in size but larger than normal.

In more advanced foci parenchyma cells are not recognizable, the ducts are more prominent, and their cells less regularly arranged, often forming multilayers about distorted lumens. Groups of these elements usually lie in loose formation with variable amounts of fibrous tissue between.

Apparently the process extends from the smaller foci to involve the whole organ, glandular tissue being replaced more or less completely by either an irregular network of ductlike structures which are supported by stroma of varying density or more compactly massed elements that resemble acini.

There appear to be two possible explanations for these lesions: Either the whole process represents primary hyperplasia of the ducts with secondary damage of the parenchyma, or the parenchyma is damaged primarily and the ductal hyperplasia is an attempt at regeneration. To me the first suggestion seems the more suitable.

NEW ENGLAND PATHOLOGICAL SOCIETY

CHARLES BRANCH, *President*

Regular Meeting, Oct. 21, 1938

GRANVILLE A. BENNETT, *Secretary*

ETIOLOGIC FACTORS IN THE DEVELOPMENT OF SILICOSIS. LEROY U. GARDNER.

While it is generally agreed that of all the inorganic dusts only free silica and asbestos are particularly harmful, there is still uncertainty about what constitutes a hazardous concentration of silica in the atmosphere. Experimental observations and observations in the field indicate that the atmospheric concentration of silica regarded theoretically as hazardous has not invariably been

associated with disease and that the amount of pulmonary reaction is not directly proportional to the quantity of silica in the rock creating a particular dust. There are two sets of factors whose action tends to retard the anticipated effects of silica; one set, acting in the atmosphere, brings about flocculation of silica with a reduction in the amount inhaled, and another, acting inside the body, retards the ordinary irritating effects of silica on the tissues. It is postulated that the latter retardation is the result of coating the grains of silica in various ways so that effective contact of silica with tissues is interrupted.

Examples of atmospheric reduction in silica from the point of dust generation to the breathing zone were shown, and the chemical and histologic effects on experimental animals were demonstrated. Evidence for the belief in atmospheric flocculation was presented.

The case for inhibitory action inside the body is based on a series of experimental observations. First, all crystalline and cryptocrystalline forms of silica excite characteristic reactions, and the rate of tissue response is inversely proportional to the particle size of the silica. The latter observation indicates a direct relation between the extent of the exposed mineral surface and the irritating properties of the mineral. Second, various silicates and nonsiliceous minerals do not produce such a reaction; in fact, most of them are essentially inert regardless of their silica content or physical properties. Third, the injection of different natural and artificial mixtures of free silica with silicates and non-siliceous minerals does not produce reactions proportional to the amount of free silica. If the mixtures contain enough free silica, a modified type of silicosis ultimately develops but at a much delayed rate. In other words, time rather than dose of silica is the determining factor. Finally, experiments with metallic aluminum and the hydroxides of aluminum and iron form the basis for the belief in a protective coating of the silica particles.

DISCUSSION

R. Z. SCHULZ: There are several questions which may be raised in connection with silicosis, its progress and the complications which may arise. The important factors in the production of the silicotic lesion have been emphasized by Dr. Gardner. Substances which may inhibit or delay the production of these lesions are of industrial and clinical importance. Erwin has advocated the use of aluminum dust as such an inhibitory substance. Limestone dust has also been used. What is Dr. Gardner's opinion concerning the role of such substances in delaying the production of the silicotic lesion? The fact that certain substances can delay the production of the lesions is also of importance to those who attempt to evaluate the hazards arising from exposure to dust in industry.

Another question I should like to ask has to do with the relation between silicosis and tuberculosis. Concerning this matter, some work has been done which indicates that when silica is added to artificial mediums for the cultivation of tubercle bacilli it has a stimulating effect on the growth of these organisms. I should like to have Dr. Gardner express his opinion concerning the possibility that silica acts as a stimulating factor in the development of tuberculosis in those human beings in whom both disease processes occur.

FRANCIS P. MCCARTHY: I hope Dr. Gardner will say something on the differentiation between silicosis and silicotuberculosis. It is of some interest to note that the pulmonary consolidation in silicosis is concentrated in the lower lobes of the lungs.

Figures pertaining to the incidence of silicosis and silicotuberculosis in patients examined at the Braintree Tuberculosis Hospital and the Quincy City Hospital indicate that silicotuberculosis is more prevalent in an older age group than is tuberculosis alone.

A questioner whose name was not obtained asked: Will Dr. Gardner say something more about inhibitor minerals?

Another question was: Apropos of the question on inhibitors, are there substances that will accelerate the progress of silicosis?

DR. LEROY U. GARDNER: In reply to a question as to the injury produced by spun glass: Injection of such material into the ear veins of rabbits produced no more reaction than the natural silicates previously described. It was therefore concluded that this material should not be considered as a dust likely to produce pulmonary fibrosis.

Several questions sought further elucidation of the action of inhibitor minerals. I believe that they merely delay the interaction between the surface of the silica particles and the body fluids; in all cases thus far observed their effect was temporary, and when observations were continued over a sufficient period silicotic fibrosis of a modified type ultimately developed. It might be possible, of course, to coat the surface of quartz grains with membranes which would never be dissolved or dislodged, and in this case permanent protection would be theoretically possible. The experiments of Kettle may be cited. He obtained no reaction to the injection of silica particles chemically coated with iron.

In discussing the possibility of substances which would accelerate the effects of silica in the tissues, doubt must be expressed as to the validity of this theory. I have not attempted to investigate such activity by experiment, but I may cite the negative evidence published by Dr. Cary McCord. He stated that in his opinion the similarity between the lesions of rapidly developing silicosis in the "soap powder cases" and those in sand blasters and workers in the Gauley Bridge tunnel pointed to an excessive concentration of comparatively pure silica in a fine state of subdivision as the probable cause of the rapidly developing disease.

All of the other questions bore on the subject of tuberculosis in association with silicosis.

In response to a question as to how to differentiate individual silicotic from tuberculous lesions, I admit the difficulty but I place much reliance on the distribution pattern of the lesions. To observe this pattern I employ large thick sections, 3 or 4 inches (7.5 or 10 cm.) in diameter, which can be studied with a low power binocular microscope. The arrangement of hyaline collagen in typical silicotic foci tends to be more uniform and presents concentric lamination, which is not found in tuberculosis. While necrosis (and even calcification) may occur in the centers of pure silicotic nodules, it is not usually associated with the leukocytic reaction. The tuberculous reaction is rarely confined to the area of nodule formation but extends into the surrounding pulmonary air spaces. In areas of conglomerate fibrosis a tuberculous complication may be manifested by cellular reaction and caseation typical of tuberculosis or sometimes merely by diffuse necrosis involving all of the preexisting silicotic reaction. In the latter case the cellular and fibrous elements lose their sharp outlines, the nuclei no longer stain, and the whole focus presents a pale ground glass appearance. Infiltration of the focus by polymorphonuclear leukocytes is usual. Stains for tubercle bacilli may or may not disclose organisms. The result of inoculation of involved tissue into guinea pigs is a more reliable criterion of infection.

Cavities may form in the centers of areas of massive fibrosis as a result of ischemic necrosis. Such cavities are different from those due to tuberculosis. Instead of being spherical or ovoid, they are generally elongated and slitlike. They do not show a wall composed of well defined layers; their margins are composed of granular debris abutting on firm, usually deeply pigmented scar tissue. They are not usually trabeculated.

In reply to a question as to the means of differentiating in the living subject between the area of massive fibrosis in simple silicosis and the lesion of tuberculo-silicosis, one must state that it is often most difficult and requires prolonged observation. The simple condition, I believe, is due to excessive accumulation of silica and other dust in pulmonary tissue damaged in most cases by previous infection. Massive fibrosis due to tuberculous complication might arise either from reactivation of an old latent focus or from reinfection in a resistant silicotic subject. Since the course of such disease is extremely chronic, without symptoms of intoxication and without tubercle bacilli in the sputum for many years, the condition might readily be confused with massive fibrosis of the first

type. However, the ultimate outcome of lesions in which living tubercle bacilli have played an etiologic role is the development of typical chronic tuberculosis with all of its characteristic signs, symptoms and tissue changes. Such an outcome might ensue only years after the first recognition of an area of tuberculo-silicosis in a roentgenogram.

The differentiation of the two lesions is based on repeated physical, roentgenographic and bacteriologic examinations of the subject. A progressive loss of body weight might be the first indication of activity. Inoculation of the sputum into guinea pigs or culture of the sputum might be necessary to detect the presence of a few tubercle bacilli. A negative tuberculin reaction is significant, provided a strong enough tuberculin is injected. (Ordinary doses frequently fail to produce reactions in men with such lesions.) Both kinds of lesions produce dyspnea, which may or may not incapacitate for work. Since treatment for such advanced lesions accomplishes little, patients should all be watched for the appearance of tubercle bacilli in the sputum. In this event they should, of course, be removed from contact with others and particularly from contact with any fellow workman who is unduly susceptible to tuberculosis by virtue of having silicosis.

CHARLES BRANCH, *President*

Regular Meeting, Nov. 17, 1938

GRANVILLE A. BENNETT, *Secretary*

PATHOLOGIC OBSERVATIONS ON EQUINE ENCEPHALITIS IN MAN. SIDNEY FARBER and CHARLES BRANCH.

This report concerns material from nine postmortem examinations at the Massachusetts Memorial Hospitals and five at the Children's Hospital, Boston, in the early fall of 1938. The diagnosis of equine encephalitis was made either by the identification of the virus obtained from the brain (L. D. Fothergill and J. H. Dingle; L. T. Webster and F. H. Wright) or by the results of pathologic examination. A composite picture of our observations follows:

Edema and congestion of the brain and spinal cord and generalized visceral congestion were present to a severe degree. On microscopic examination diffuse meningoencephalomyelitis was found. The changes may be regarded as of several types. First, there was widespread involvement of nerve cells ranging from early involvement of nuclei and cytoplasm to complete disappearance of these. The areas of dead and dying neurons contained cellular collections composed mainly of polymorphonuclear cells and microglial cells. The second type of change consisted in perivascular accumulations of cells. In the early stages of the disease this reaction consisted mainly of neutrophils, lymphocytes and large mononuclear cells. As the duration increased, the predominant cells were lymphocytes and large mononuclear cells, a cell picture which paralleled that in the spinal fluid. Third, there was diffuse meningitis, most marked over the base of the brain and present only to a slight degree over the cord. The meningeal reaction corresponded to that found in the perivascular spaces. One instance was noted in which the meningeal reaction over the base of the brain was severe enough to give the gross picture of acute pyogenic meningitis. The fourth type of characteristic reaction consisted in the presence of lesions in both arterioles and venules. Numerous small thrombi were present in the small vessels. In addition, many of the vessels showed complete involvement of their walls, characterized by neutrophilic infiltration and deposition of fibrin actually within the walls. Demyelination was not a prominent feature except in areas where tissue had been destroyed entirely by the inflammatory process.

The cord in most of the cases showed no involvement except for edema and congestion. Occasional evidences of early damage to nerve cells could be demonstrated. One case was noted in which the entire cord showed evidences of nerve cell destruction.

The distribution of the lesions was of some interest. The most severe involvement was found in the basal ganglions and in the brain stem. There was diffuse involvement of the cortex, although numerous small uninvolved areas could be found. The olfactory bulb was either spared or little involved. The cerebellum showed occasional slight evidences of inflammation, and these corresponded in degree of severity to those found occasionally in the cord.

This type of encephalitis falls into Greenfield's group II of polioclastic encephalitis. In this group are found encephalitis lethargica, rabies, equine encephalomyelitis, St. Louis encephalitis, Japanese encephalitis B and poliomyeloencephalitis. The picture in equine encephalitis as seen in this material corresponds closely to that described in St. Louis encephalitis and Japanese encephalitis B. Qualitatively the changes are similar to those found in encephalitis lethargica; the nearest comparison, which amounts in some cases almost to duplication, particularly after the disease process has reached the five day stage, is that to the picture in St. Louis encephalitis. Vascular lesions, which were so prominent in these instances of equine encephalitis, have not been stressed in the other forms. Except for the difference in distribution, with particular reference to the absence of involvement of the anterior horn cells in the spinal cord in this disease, the type of damage and cellular response closely corresponds to that observed in poliomyeloencephalitis. No bacteria were found in association with the early lesions, either by cultural or by staining methods.

The changes in the body consisted mainly of congestion and edema. Severe pulmonary edema was present as a terminal phenomenon in all cases. In the lungs of a patient who died forty-eight hours after the onset and from whose blood stream no organisms could be cultured, early interstitial pneumonia was found. In various organs of the body of the same patient small vessels showed scattered small thrombi.

A detailed report of the pathologic observations is being prepared.

DISCUSSION

ALLEN M. HILL (by invitation): During a period of six weeks, beginning late in August and ending early in October, there was admitted to the Children's Hospital a group of 8 patients who, by the character of their illnesses, the physical findings and the results of studies of the spinal fluid, appeared to be suffering from a severe form of encephalitis. To date 5 of these patients have died, and 3 have recovered sufficiently to be able to return home. All of those who succumbed were examined post mortem, and from 3 a virus was recovered which was identified as that of the Eastern strain of equine encephalomyelitis. A study of the serums of those who recovered, in which neutralization tests are being made, has not as yet been completed.

The ages of the patients ranged from 1 month to 7 $\frac{1}{2}$ years. Seven were infants under 18 months of age.

In none of the patients did the illness progress for a period of more than forty-eight hours before admission to the hospital. The onset was abrupt, with all the patients becoming acutely ill within three to twelve hours. Each presented on admission to the hospital two or more of the following symptoms: high fever, vomiting, coma, rigidity and convulsions. At one time or another during the illness all had one or more generalized tonic, and occasionally clonic, convulsions. Neurologic examination revealed Kernig's signs, mild cervical rigidity and semiconsciousness or coma, and in infants, tense bulging of the fontanel. In those who survived sufficiently long, a peculiar nonpitting edema, prominent in the periorbital spaces and to a lesser extent in the extremities, developed three to four days after the onset of the illness. Leukocytosis was present in all, the cell count ranging from 13,600 to 35,000, with the differential

count showing from 64 to 90 per cent polymorphonuclear leukocytes. The spinal fluid (on admission) showed from 246 to 2,000 cells, with polymorphonuclear leukocytes predominant in percentages of 60 to 100. Within a few days the spinal fluid cell count decreased, and this decrease was accompanied by a reversal in the polymorphonuclear leukocyte-mononuclear cell ratio. The protein of the spinal fluid was elevated in all (50 to 140 mg. per hundred cubic centimeters), the sugar content was normal (qualitative), and cultures gave no bacterial growth. A search for tubercle bacilli was unsuccessful.

Of the patients who died, 2 succumbed within forty-eight hours after the onset of the illness, 2 lived seven and nine days, respectively, and 1 died at the end of twenty-one days. Of the 3 children who survived, all were found to have some neurologic residual: 1, spastic right hemiplegia and partial deafness; 1, right hemiparesis and incoordination of the upper extremities, and 1, spastic diplegia. There has been some degree of improvement in all these patients since their return home.

LEROY D. FOTHERGILL (by invitation): The clinical features, epidemiologic aspects and mode of transmission of the Eastern and Western varieties of equine encephalomyelitis were reviewed. During the past summer an extensive epidemic of encephalomyelitis occurred among horses in southeastern Massachusetts. Encephalitis appeared in a number of human beings in the area where the horses were diseased and was shown to have been caused by the virus of the Eastern variety of equine encephalomyelitis. This virus was isolated from the brain tissue in 8 fatal cases. Its identity was established by protection tests in immunized guinea pigs and by neutralization tests with hyperimmune serums.

During this epidemic a total of about 40 probable cases in man came under observation. The mortality rate was about 65 per cent. At the present time, extensive studies are being conducted by means of neutralization tests. The serums of convalescent patients, of various contacts of the patients, of veterinarians and of various other persons, such as those suffering from minor illnesses, are being examined. Clearcut positive results are being obtained with the serums of convalescent patients. Neutralizing antibodies have not been demonstrated in the serums of family contacts or in veterinarians so far as concerns the specimens examined up to the present time. A detailed report of these studies will be published in the near future.

JOHN H. DINGLE (by invitation): I have but one or two comments to add. The virus of equine encephalomyelitis has been widely used in investigative work, not only because there is little danger of laboratory infection in man, as Dr. Fothergill pointed out, but also because the common laboratory animals are susceptible to it. In addition, experimental infections have been produced by Remlinger and Bailly and by others in various avian species. No such infection, however, has been produced in the chicken.

In 1933 Giltner and Shahan produced infection in pigeons with the Western type of the virus. These workers, as well as Ten Broeck, Hurst and Traub, pointed out the possibility that birds were host reservoirs.

During the recent epidemic here the death of unusual numbers of pigeons was noted by dealers and residents in the region of the epidemic and in surrounding areas. My co-workers and I were fortunate in obtaining one of these pigeons, and from the brain we isolated a virus which we identified as the virus of the Eastern type of equine encephalomyelitis. This virus, as well as the strain obtained from human brain material, produced a fatal infection in normal pigeons.

This observation indicates that the domestic pigeon may be a host reservoir of the virus; indeed, it is possible that these birds may constitute a primary reservoir and that both horses and man are secondary hosts. If such is the case, the observation mentioned by Dr. Fothergill, that the disease in horses appeared almost simultaneously on both sides of Chesapeake Bay, is easily explained. In this regard it is interesting that one of the pigeon dealers noted that the "losses of pigeons stopped with the onset of cold weather"—an observation which bears out what has been repeatedly observed in epidemics of the disease in horses.

NEW YORK PATHOLOGICAL SOCIETY

ALFRED PLAUT, *President*

Nov. 18, 1938

ROBERT A. MOORE, *Secretary*

ADENOCARCINOMA OF THE JEJUNUM TREATED BY ENTEROENTEROSTOMY AND COMPLICATED BY INTUSSUSCEPTION. LESTER M. FRIEDLAND (by invitation) and M. F. WIENER.

A white man aged 68 was admitted to the Cumberland Hospital, Brooklyn, on Feb. 10, 1938, with a history of having lost 20 pounds (9.1 Kg.) in weight within the past four months and a complaint of abdominal pain, vomiting and constipation, increasing in severity and frequency, for the past ten days. The past history of the patient and the family history were irrelevant. The patient was cachectic; the temperature was 101.6 F.; the pulse, 106; the respirations, 26, and the blood pressure, 118 systolic and 64 diastolic. The heart and lungs were normal. The abdomen was soft and distended. There was visible peristalsis in the right half of the abdomen. No masses were felt.

The blood showed moderate polynucleosis and an increased urea nitrogen content (34 mg. in 100 cc.). The gastric analysis gave normal values. Roentgen examination showed distention of the intestines.

The patient was observed until February 11, when, under local anesthesia, an exploratory laparotomy was made through a midline incision. A hard annular constriction of the jejunum producing intestinal obstruction was discovered. A diagnosis of carcinoma of the jejunum was made. Because of the poor condition of the patient, resection was deferred, and a side to side anastomosis short-circuiting the constricted portion was performed.

There was marked improvement for forty-eight hours after the operation, followed by complicating parotitis and abdominal pain, distention and tenderness, and finally shock, from which the patient died on the twelfth postoperative day.

Autopsy.—The abdomen showed a clean laparotomy wound. The left inguinal region bulged and was found to contain gas communicating with that in the peritoneal cavity. The peritoneal cavity contained about 2,000 cc. of purulent fluid and foul-smelling gas. All surfaces of the intestine were covered with fibrin and were adherent. The heart weighed 460 Gm. The lungs showed bronchiectasia, pleural thickening, multiple calcified pleural nodules and bilateral apical fibrosis, with surrounding areas of caseation and calcification.

About 1.2 meters from the duodenojejunal junction was a large mass consisting of intussuscepted jejunum. When the outer layer was opened, the mucosa presented itself in the shape of a tube 30 cm. in length. The lumen of the distal end of this was markedly constricted by a fungating, partly necrotic and ulcerated annular adenocarcinomatous mass. Ten centimeters proximal to the mass a slit-like stoma of the recent anastomosis communicated with the internal cylinder of intestine. At one end of the stoma there was a perforation of the wall, 8 mm. in diameter, communicating with the peritoneal cavity.

The liver showed fat replacement and mild cirrhosis. The kidneys showed arteriosclerotic changes and extratubular deposition of calcium. The prostate was hypertrophied.

Anatomic Diagnosis.—The following conditions were diagnosed: carcinoma of the jejunum; a jejunojejunostomy wound; intussusception of the jejunum; perforation of the jejunum (operative site); generalized peritonitis; benign hypertrophy of the prostate; hydronephrosis on the right; renal arteriosclerosis and calcinosis; apical pulmonary tuberculosis (healed); nodular calcification of the

pleura; bronchiectasis; fibrous pleural adhesions; cardiac hypertrophy; replacement of the liver by fat; visceral congestion and cloudy swelling.

Carcinoma of the jejunum is rarely encountered, the largest series reported being that from the Mayo Clinic, which comprised 21 cases recorded up to 1929. A review of the literature to date fails to reveal any case similar to that described here.

The probable pathogenesis of the intussusception and the anatomic relations of the tumor and the enteroenterostomy are conceived to be as follows: The tumor produced obstruction, which was relieved by the short-circuiting entero-anastomosis. The latter was sufficiently near the neoplasm to be involved in the intussusception, which probably took place ante mortem.

Summary.—A thorough review of the literature reveals the infrequency of carcinoma of the jejunum. A case of this condition is reported here. The patient was treated by enteroanastomosis. This was followed by perforation, generalized peritonitis and preterminal intussusception.

SUPPURATIVE THROMBOPHLEBITIS OF THE AZYGOS VEIN ASSOCIATED WITH ABSCESS OF THE LUNG. JACOB FURTH.

Thrombophlebitis of the azygos vein is of importance from the standpoint of the pathogenesis of septic infections and their surgical control. There are only 2 cases of this disturbance on record, one reported by H. Nathan (*Virchows Arch. f. path. Anat.* **281**:430, 1931) and the other by E. Beer (*Ann. Surg.* **96**:687, 1932).

Our patient, a 57 year old man, acquired in 1936 an abscess of the lung, the etiologic background of which was not disclosed. Following resection of a rib and drainage of the abscess, he was well until five weeks after the operation; then pain appeared in the region of the fifth rib, and the temperature rose. Two months after the operation an abscess was noted in the wall of the chest and was drained. Ten weeks after the operation the patient died.

Postmortem examination showed fibroid pneumonia involving the greater part of the middle lobe of the right lung and adjacent parts of the upper and lower lobes, purulent bronchitis with slight bronchiectasis, and bronchopneumonia of all lobes. A small cavity, lined by thick fibrous connective tissue, marked the site of the drained abscess. Pleural adhesions bound the lungs to the chest wall. There were numerous abscesses in all lobes of the lung, most of them located peripherally. The posterior mediastinum was the site of suppurative inflammation with extensive fibrous adhesions and numerous pockets of pus. The azygos vein was embedded in this fibropurulent mass of tissue. Adherent thrombus filled the lumen of the distant third of the vein, and its remaining part was almost completely occluded by fibropurulent material. Friable red thrombi were seen in several small branches of the pulmonary artery, but the pulmonary veins appeared free from thrombi. The other organs were free from abscesses. A smear of the exudate of the azygos vein contained numerous mononuclear and polymorphonuclear leukocytes and many long chains of streptococci. The sputum contained hemolytic *Staphylococcus aureus* and nonhemolytic streptococci of the alpha type. The pulmonary abscess, pus from the azygos vein and blood from the right auricle of the heart, taken post mortem, yielded nonhemolytic alpha streptococci. Blood taken from veins of the arm during life and cultured was sterile.

The findings are in accord with the views of Schottmüller and Nathan on the pathogenesis of sepsis. In Nathan's case there was, in addition to thrombophlebitis of the azygos vein, thrombophlebitis of the pulmonary vein, and accordingly there were numerous abscesses in the greater circulation, including the kidneys and liver. In the present case only the pulmonary arteries were involved, and abscesses were not found in the organs supplied by the greater circulation.

IMMUNOLOGIC STUDIES WITH TRANSPLANTABLE AND VIRUS-INDUCED GROWTHS OF RABBITS. JOHN G. KIDD (by invitation).

An immunologic study of the Shope papilloma has shown that saline extracts of the growths contain a complement-binding antigen that is closely associated, if not identical, with the virus causing them (*J. Exper. Med.* **68**:703, 725 and 737, 1938). A similar study of the transplanted Brown-Pearce carcinoma has demonstrated that it, too, contains a specific complement-binding antigen, which is similar in its general traits to that derived from the virus-induced papillomas (*Proc. Soc. Exper. Biol. & Med.* **38**:292, 1938).

The antibody that reacts with the complement-binding antigen of the Brown-Pearce tumor has been found only in the serum of rabbits which bear the Brown-Pearce tumor or in which this tumor has retrogressed. It has not been present in the serum of the many normal rabbits tested, nor in the serum of rabbits with transplanted uterine cancers (Greene), of rabbits with virus-induced papillomas or of rabbits with various other diseases, including syphilis and vaccinia. The complement-binding antigen has regularly been found, and in considerable amount, in saline extracts of the Brown-Pearce tumor, wherever this is situated in the body. It has not been present, however, in extracts of the normal tissues of rabbits, in extracts of their uterine cancers or virus-induced papillomas or in extracts of rabbit tissues infected with vaccine virus or virus III.

Heating at 60 C. for thirty minutes has no great effect on the complement-binding antigen of the Brown-Pearce tumor, but heating at 65 C. inactivates it, as does also treatment with alcohol, treatment with acid to p_H levels of 2.5 and 4.5 and treatment with alkali to p_H levels of 10 and 11.5. In three experiments the antigen has passed readily through membranes with average pore diameters of 471, 397 and 383 microns and larger, but not through others with pore diameters of 348, 293 and 255 microns and smaller. The active material can be deposited readily with the high speed centrifuge, all of it coming down at 20,000 revolutions per minute for ninety minutes. The active material when purified by four differential centrifugations gives strongly positive results in the Millon, xanthoproteic and biuret tests for protein and a delayed Molisch reaction (immediately positive on hydrolysis). Spectroscopic examination of the purified active material from several sources has shown in each instance characteristic curves, with maximum absorption in the region of 2,600 angstroms (Lavin).

(The possible significance of the findings was discussed.)

DISCUSSION

JACOB FURTH: Dr. Kidd has convinced those present that both the papilloma and the Brown-Pearce carcinoma contain heavy substances, presumably proteins, with similar properties. I suppose, although he did not mention this, that one produces tumors and that the other is innocuous. I should like to hear about his attempts to produce tumors with the heavy protein of the Brown-Pearce tumor.

JOHN G. KIDD: So far as the experiments go, the antigenic material derived from the Brown-Pearce tumor has not proved pathogenic. The work has not gone far enough, however, to be conclusive.

JOEL WARREN: Is there any difference in the complement-fixing power of an extract of the papilloma of the domestic rabbit in contrast to the complement-fixing power of an extract of the growth in the cottontail?

JOHN G. KIDD: Extracts of growths from domestic rabbits, which contain little or no active virus, fix complement poorly or not at all. Extracts of papillomas from cottontails, containing much virus as a rule, fix it notably well.

JACOB FURTH: There is a minor question of importance to those who work in this field. The tables show that the titer of complement fixation was 1:320 with the crude extract, but only 1:40 with the virus concentrate obtained by high speed centrifugation, while the supernatant fluid was inactive. One would expect

the concentrated virus to be more active than the crude extract. How does Dr. Kidd explain this finding?

JOHN G. KIDD: The sedimented material used in the test was not concentrated; it was finally resuspended in the same volume of fluid as was present originally. I have a feeling that some of the virus and some of the complement-binding antigen remained in the supernatant fluid and stuck to the sides of the tubes in this experiment, as it undoubtedly has in other similar tests, and hence the amount of active material in the sediment was reduced in comparison with the crude material. Furthermore, if one throws material down hard in a centrifuge there is inevitably a good deal of aggregation of the sedimented stuff; not all of it can be resuspended in monodispersed form; much remains in clumps, and the clumped material is not very effective as a complement-fixing antigen. All these incidents, perhaps, account for the finding mentioned.

BIOPSY OF MATERIAL OBTAINED BY ASPIRATION IN THE DIAGNOSIS OF TUMORS.
FRED W. STEWART (by invitation).

It is now eight years since diagnostic aspiration for the detection of neoplasms was first practiced in the Memorial Hospital for the Treatment of Cancer and Allied Diseases. Members of the staff claim no originality for their use of the method but only a rather extensive experience in the application of this very simple procedure. The exact number of cases in which a diagnosis of cancer was made by this method has not been determined, but in the past four years there have been upward of 2,600 cases in which a positive diagnosis of cancer has been rendered from material obtained through a 17 or 18 gage needle. The aspiration biopsy is never employed in cases of an accessible ulcerated tumor but only in cases in which either no tissue for biopsy could be obtained otherwise or in which circumstances make surgical excision of tissue for biopsy undesirable. The technic for obtaining material has already been described, and its exact fulfilment contributes notably to success in the use of the method. A negative report is of little significance and may be regarded as of value only when the clinical evidence is fully compatible with such a report. In the early days of the use of the method my associates and I viewed it with some hesitancy, a hesitancy fully shared by certain colleagues, who became vociferous in their objections. In our opinion, the method is becoming more useful year by year, and, properly interpreted, the smear is as valuable as the section. Naturally there are definite limits to interpretation of a smear, but one has to accept a method in the light of its usefulness rather than in that of its limitations.

One might refer to interpretation of a section as analytic pathologic examination. Interpretation of a smear, on the contrary, might be called synthetic pathologic examination, since one has to reconstruct the organized pathologic picture from loose cells and small aggregates of cells and rarely from one cell. To do so one must know all the clinical data in the case and especially the exact history of the patient and the status of the part subjected to aspiration of material. One cannot merely look at a smear and expect an immediate diagnosis of some specific type of cancer. If, however, one knows the age of the patient, the region involved and the history of the illness, and if one possesses knowledge of the types of tumors, benign and malignant, common to the region, one can from the isolated cells of a smear synthesize the pathologic picture and arrive at a proper diagnosis. (A demonstration of lantern slides followed.)

DISCUSSION

N. CHANDLER FOOT: I do not know why there has been so much "sales resistance" to the presentation of this method, and I think that after hearing Dr. Stewart's presentation tonight most of those present will see that there is nothing formidable about the method and that it ought to be employed much more than it is. It is being used at New York Hospital, with very good success, but

there has always been a disinclination on the part of the surgeons to use this method rather than biopsy of tissue obtained by excision. The only tumors with which my associates and I have had difficulty have been those of the stringy, fibrous, rather dry type sometimes encountered in the chest in the form of desmoid fibroma or of fibrosarcoma, which is well differentiated and very fibrous. Most of the other malignant tumors that we are interested in are more or less soft and apt to be granular, and there is not much difficulty in getting the material into the syringe. Dr. Stewart has shown the method. I think if one explains to the surgeon that if the vacuum is maintained, most, if not all, of the tumor cells that are detached will go into the syringe and not be transmitted elsewhere in the body, his fear of metastases will be allayed. In my opinion, there is not much ground for this fear. All the members have seen that most of these slides, even when shown as black and white lantern slides, are fairly readily diagnosed from their seats—one was so difficult that Dr. Stewart himself was not quite sure about it—but when a pathologist looks at these in the laboratory he knows where they come from, the surgeon has told him the source of the aspirated material and the history of the patient, and there are a great many other aids, among them that of color.

This leads me to the matter of stains. One can use a variety of stains; one is not limited to hematoxylin and eosin, and I think in studying material aspirated from lymph nodes one arrives at definite impressions, particularly in cases of Hodgkin's disease, if one uses a good Giemsa stain, for instance, and is fortunate in finding eosinophils in the aspirated material, which one usually does.

The interpretation of the evidence of tumor is sometimes difficult, but I believe that most of the difficulty is encountered in the early days of one's experimentation with the method. After one has experimented with it a little while, one acquires a good deal of insight and has no difficulty in making a satisfactory diagnosis. In the case of tumor of the breast in which an aspiration biopsy and later a radical operation have been done, it is always possible to have a biopsy of excised tissue at the time of operation, as a check up, and it is also possible to examine material obtained in the axillary dissection and see whether or not metastases have occurred there. So I see no objection to this method at all, and feel that it is not used as often as it should be.

A. R. CASILLI: My associates and I have a modest experience with the aspiration biopsy and our efforts have been more than compensated by the success we have had with it. A successful reading of the aspirated material depends on several factors. One of them is that the pathologist should have familiarized himself with the appearance of various tumor cells, and the best way to do that is to aspirate material from surgical specimens as a routine and study the cell pictures presented. A second factor is the eosin and hematoxylin stain, which is of fundamental importance. The third—and this offers an advantage over the ultimate paraffin section—is that with most aspiration biopsy slides one can reach a diagnosis within a short period, perhaps ten or fifteen minutes, after the time of aspiration.

AMOUR F. LIBER: What criteria are used in judging whether mucin is present? Are special stains necessary?

FRED W. STEWART: No.

AMOUR F. LIBER: How do you differentiate mucin and interstitial substances, such as those found in myxoid degeneration?

FRED W. STEWART: We are making diagnoses of cancer from smears; we are not interested in the specific nature of mucoids.

SOLOMON WEINTRAUB: My associates and I have not been fortunate in getting surgeons to use the aspiration biopsy, but internists have permitted us to make biopsies of this type on enlarged livers. Our method is somewhat different from that of Dr. Stewart. I wonder if he has used the method which we use. We

take a syringe containing physiologic solution of sodium chloride and equipped with a 13 gage needle. After we have struck the liver, we aspirate into the saline solution and remove the whole content with the needle. In that way we have been able to get enough material for diagnosis in every case. I wonder whether the saline method has been used at the Memorial Hospital.

FRED W. STEWART: We have not used it. We find the 18 gage needle perfectly satisfactory, and we want to keep the procedure as simple as possible. Just as soon as people began to write about the method, there appeared all kinds of complicated gadgets with which to get the tissue, and outfits were manufactured and sold for \$25, whereas any tight needle and syringe can be used. We have always used the technic that I described, which can be carried out in any one's office.

Book Reviews

La réaction de fixation dans les tuberculoses humaines et animales. Achille Urbain. Second edition. Pp. 146. Price 28 francs. Paris: Masson & Cie, 1938.

The publication of this second edition of "Complement Fixation in Tuberculosis" was delayed by the necessity of completely revising the first edition. Although certain chapters were maintained within their primary limits, to others extensive additions were made, necessitated by the recent advances in this field. The volume is further augmented by a complete bibliography of the subject and an enlightening historical introduction. No detail, practical or theoretical, is omitted. If one wishes to read only about an interesting branch of scientific achievement, recorded from its inception at the turn of the century, this volume supplies both enlightenment and entertainment. The text is composed of seven chapters, beginning with the preparation of the elements of the reaction and their titration, and no details are lacking as to their preparation, the essential systems and finally (chapter 3) the technics of the reaction, in the presentation of which the methods of Calmette and Massol, Besredka and Petroff are elucidated. An additional chapter (4) is devoted to the tuberculous antibodies, their presence in organs and exudates, their transmission from mother to infant, the cutaneous reactions and tuberculous antibodies, the nature of these antibodies and their role. Chapter 5 is devoted entirely to complement fixation and the Vernes resorcinol seroflocculation and a comparison of the results of the two technics. The extensive studies in the field of diagnosis by complement fixation in tuberculosis become evident (chapter 6) only when the results in the various forms of localization of the disease (visceral, lymphatic, osteoarticular, cutaneous and seromembranous) are detailed. Finally, a chapter (7) is devoted to the application of complement fixation to the diagnosis of tuberculosis in animals. After completing the text, which is written in French, one cannot go far astray in concluding that this volume of 146 pages should find a place on the bookshelf of every serologist who can read French, as a ready reference to any phase of complement fixation in tuberculosis. It should be available also to every student of tuberculosis and medicine who faces the problem of being conversant with the various diagnostic phases of tuberculosis. Even though the application of fixation to the diagnosis of human tuberculosis is still disputed and the best figures admit only from 85 to 95 per cent positive reactions in cases of pulmonary tuberculosis, with 10 per cent positive reactions among nontuberculous persons, there are those who maintain that with proper reservations and discriminative evaluation the method is useful and of real interest in medicine when considered in the light of the results of other methods of examination. Among animals, the reaction is positive with from 90 to 98 per cent of those that have tuberculosis and negative with from 90 to 100 per cent of those that are not tuberculous. As a whole, this compact volume by Urbain is a good presentation of a difficult subject in a highly specialized field of medicine.

Surgical Pathology of the Diseases of the Neck. Arthur E. Hertzler, M.D., Surgeon to the Agnes Hertzler Memorial Hospital, Halstead, Kan., and Professor of Surgery in the University of Kansas. Pp. 237, with 206 illustrations. Price \$5. Philadelphia: J. B. Lippincott Company, 1937.

This is the latest in a series of nine monographs on surgical pathology, with others to follow. The previous monographs dealt, in the order of their appearance, with diseases of bones, of the skin, of blood vessels, of muscles and nerves,

of the genitourinary tract, of the female generative organs, of the mammary glands, of the peritoneum, of the gastrointestinal tract and of the thyroid. Each dealt with an organ or a group of organs that functionally formed a natural unit.

The subject matter of the latest monograph is a region of the body with a variety of organs united only topographically. That deprives the book of some of the coherence which characterized the former monographs.

It has, as have the former monographs, the distinctive flavor of the author's personality in its style, in its humor and even in the treatment of the subject matter. This personality is the same that is responsible for the already apparent literary success of the recent autobiography of Hertzler.

The first chapter deals with a general review of surgical conditions of the neck; the following three chapters take up Hodgkin's granuloma, lymphosarcoma, lymphoepithelioma and endothelioma of the lymph nodes (which Hertzler persists in calling lymph glands). Rare primary tumors (of the carotid body, of the nerves and so forth) and tumors due to developmental abnormalities are discussed in the fifth and sixth chapters. The remaining four chapters have to do with benign tumors, with inflammations and tumors of the salivary glands, with metastatic growths and with inflammatory lesions of the neck.

The book will be of considerable interest to the surgeon and ought to provide pleasant reading for the pathologist, although the informed pathologist will find in it relatively little factual information.

The illustrations are abundant and excellent; the photographs of specimens and especially the photomicrographs would gain if the magnification were stated.

The bibliographic references are intended to be selective. The index fills 9 pages and is well organized. The paper, print and binding are excellent.

La tuberculose pulmonaire chez les sujets apparemment sains et la vaccination anti-tuberculeuse. L. Sayé, Professor de Phthisiologie à l'Université de Barcelone. Paper. Pp. 256, with 88 illustrations. Price, 60 francs. Paris: Masson & Cie, 1938.

This timely monograph on the occurrence of tuberculosis among apparently normal persons summarizes the numerous publications of recent years dealing with the epidemiology, pathology, diagnosis, therapeutics and prophylaxis of tuberculosis. There is definite evidence that the rate of tuberculization in urban communities is falling. However, roentgenologic demonstrable pulmonary lesions of tuberculosis have been found in from 1 to 3 per cent of persons apparently well. The incidence of tuberculosis among soldiers, students, nurses and other groups is summarized. Of the newer problems in the field of pathology, the occurrence and the mode of production of epituberculosis are discussed. Sayé concludes that epituberculosis results from compression of bronchi by enlarged nodes. This condition is at times associated with the presence of tubercle bacilli in the sputum. The relative importance of physical, fluoroscopic and roentgenographic examination, the importance of the tuberculin test and the demonstration of tubercle bacilli are discussed in the chapter dealing with the diagnosis of tuberculosis. Sayé considers fluoroscopic examination important but inferior in value to roentgenographic examination. In his discussion of allergy he mentions that the intracutaneous test has greater sensitivity than the Pirquet test. In his own investigations he has used Koch's old tuberculin in amounts up to 10 mg. With the purified protein derivative of the tubercle bacillus the reactions were less marked than with old tuberculin, but the percentage of positive reactors was the same with both tuberculins. The result obtained by microscopic examination of sputum and gastric contents for tubercle bacilli is inferior to that obtained by either cultural procedures or inoculation of animals. Sayé devotes considerable space to the use of the BCG vaccine. He is convinced of its innocuousness and its value in protecting against tuberculosis and believes that this vaccine will play an important role in the control of tuberculosis. In conclusion he discusses the

importance of periodic examination of contacts. The style is terse, and the numerous tables summarizing the results of various investigators are highly informative.

A Textbook of Bacteriology. Thurman B. Rice, A.M., M.D., Professor of Bacteriology and Public Health at the Indiana University School of Medicine. Second edition. Cloth. Pp. 563, with 121 illustrations. Price \$5. Philadelphia: W. B. Saunders Company, 1938.

In the preface Rice expresses himself as feeling "that the book has been highly successful." The present revision retains the plan of the first edition, namely, that of providing a relatively simple textbook of such size that students may be expected to master it in one semester. The book is intended mainly for the medical student. It covers bacteriology and the applications of the science to practical medicine in considerable detail, and it may well be doubted whether students really can "master it in one semester." But all the topics of the 58 chapters are not of equal importance to the student, and there is room for condensation and even elimination if "the textbook should contain only those phases of the subject as are rather definitely established." By way of illustration reference may be made to the discussion of "antivirus" on page 475 and elsewhere. Here and there a tendency to dogmatism has resulted in pronouncements the soundness of which may be questioned. For example: Is there reliable evidence that the Semple vaccine is of value in preventing rabies in dogs? Is it established that "aggressins" and "antiaggressins" exist as specific substances? Is it accepted that human convalescent serum is of value in the treatment of infantile paralysis? Is it known that foreign proteins "after being precipitated, are phagocytized and in that way digested so that they can no longer do harm?" Is it sound practice to permit or arrange for children to have measles "when they can be given proper care"? Does any one know that there is increased susceptibility to infection in the "negative phase"? Is it definitely settled that tetanus toxin is not absorbed by any other cells than nerve cells and that it is not conveyed to the central nervous system by the blood? No mention is made of lymphocytic choriomeningitis, venereal lymphogranuloma and the Frei test or of the remarkable recent advances in the investigation of yellow fever. The omission of all bibliographic references robs the independent student of guidance to outside reading and tends to lower the scientific level of the presentation.

The Harvey Lectures Delivered under the Auspices of the Harvey Society of New York, 1937-1938. Under the patronage of the New York Academy of Medicine. Series XXXIII. Cloth. Pp. 275, with illustrations. Price \$4. Baltimore: Williams & Wilkins Company, 1938.

This volume contains the following lectures: The Nature of the Visual Process, by Selig Hecht, professor of biophysics, Columbia University; the Pasteur-Meyerhof Reaction in Muscle Metabolism, by Einar Lundsgaard, professor of physiology, University of Copenhagen; The Functional Significance of the Lymphatic System, by C. K. Drinker, professor of physiology, Harvard University School of Public Health; Transfer of Water and Solutes in the Body, by John P. Peters, professor of medicine, Yale University; The Cortical Representation of Somatic Sensibility, by Philip Bard, professor of physiology, Johns Hopkins University; The Isolation and Properties of Tobacco Mosaic and Other Virus Proteins, by Wendell M. Stanley, the Rockefeller Institute for Medical Research, Princeton, N. J.; The Chemistry and Biology of Male Sex Hormones, by F. C. Koch, professor of biochemistry, University of Chicago; Experimental Hypertension Induced by Renal Ischemia, by Harry Goldblatt, professor of experimental pathology, Western Reserve University. These lectures review ably recent advances in the fields of research represented.

A Critical Investigation of the Blood Groups and Their Medico-Legal Application. Dawood Matta, M.B., Ch.B. (Cairo), Ph.D. (Glasgow), Lecturer on Forensic Medicine, Faculty of Medicine, University of Egypt, Cairo. Pp. 231. Cairo: The Egyptian University, 1937.

The main thesis of this study is the demonstration of the existence in groups B and AB of subgroups analogous to the subgroups of groups A and AB. On this basis human beings could be divided into the following blood types: O, A₁, A₂, B₁, B₂, A₁B₁, A₁B₂, A₂B₁ and A₂B₂. Matta has produced a number of anti-O reagents by suitably diluting and absorbing immune serums obtained by injecting human blood of group O into certain rabbits and goats. Such serums, according to Matta, act intensely on all O bloods, somewhat less strongly on bloods of subgroups A₂ and B₂, only moderately on blood of subgroup A₂B₂ and weakly or not at all on bloods of the other subgroups. The author asserts that the published exceptions to Bernstein's theory are probably valid. He therefore proposes a new hypothesis to explain the inheritance of the blood groups and to account for the existence of the subgroups. However, there are weaknesses in his arguments against Bernstein's theory and obvious flaws in his own theory of inheritance. The inheritance of the blood groups, subgroups and M-N types was studied in a series of 104 families with 315 children, and no exception was encountered to Bernstein's theory of the inheritance of the blood groups or to the theory of the inheritance of the M-N types held by Landsteiner and Levine. Matta also describes some of his experiences in determining blood groups on dried blood stains and secretions.

Human Pathology. Howard T. Karsner, M.D., Professor of Pathology, Western Reserve University, Cleveland, Ohio. With an Introduction by Simon Flexner, M.D. Fifth edition, revised. Cloth. Pp. 1013, with 461 illustrations. Price, \$10. Philadelphia: J. B. Lippincott Company, 1938.

The first edition of this book was published in 1926. The purpose of the work was and is "to present the morphologic alterations incident to disease in the light of modern views as to their functional significance." The issue of a new and revised edition approximately every third year shows that the book has met a definite need. The present edition presents a thorough revision of every chapter, new illustrations and new references. No change has been made in the general arrangement of the book. Human pathology is presented in the two traditional divisions of general and special. The field usually covered by general pathology has been reduced somewhat, and wisely so, by limiting the chapter on infection to general principles. The author writes (page 219): "The phenomena of resistance and immunity are covered in special texts. The pathology of many of the special infections must be sought in the sections on special pathology or in books and original articles on the subject." The division of special pathology, as customary, includes all the organ systems except the skin, eye and ear. Karsner's book is well arranged and well written; it will be a reliable guide for the student and useful for reference.

A Textbook of Histology. Alexander A. Maximow, late Professor of Anatomy, University of Chicago, and William Bloom, Associate Professor of Anatomy, University of Chicago. Third edition. Cloth. Pp. 668, with 542 illustrations. Price \$7. Philadelphia: W. B. Saunders Company, 1938.

This book needs no detailed introduction. It has achieved the position of a standard text, noted for the competence and clearness of its presentation and the excellence of its illustrations. The present edition has been revised thoroughly and includes the recent advances in histology and "histophysiology." In the explanation of the relation of function to structure, facts are taken from pathology as well as from physiology. Forty-two new illustrations have been added, including one in colors of the cells of the human marrow. It is a pleasure to add that the publisher has done his part most creditably.

Books Received

ANNUAL REPORT OF THE MEDICAL DEPARTMENT FOR YEAR ENDED 31ST DECEMBER, 1937, INCLUDING THE ANNUAL REPORT OF THE MEDICAL LABORATORY, DAR ES SALAAM, TANGANYIKA TERRITORY. Paper. Pp. 92. Price 4 shillings. Dar es Salaam: Government Printer, 1938.

REPORT ON RADIUM BEAM THERAPY RESEARCH 1934-1938. Constance A. P. Wood, L. G. Grimmet, T. A. Green and others. Medical Research Council Special Report Series, no. 231. Pp. 75. Price \$1.20. London: His Majesty's Stationery Office, 1938.

HANDBUCH DER VIRUSFORSCHUNG. Herausgegeben von Prof. Dr. R. Doerr, Basel, and Prof. Dr. C. Hallauer, Berne. Erste Hälfte. Paper. Pp. 546, with 71 illustrations. Price 66 reichsmarks. Vienna: Julius Springer, 1938.

ÉTUDE MORPHOLOGIQUE ET BIOLOGIQUE SUR LES FLAGELLÉS INTESTINAUX PARASITES DES MURIDÉS. ÉTUDE COMPARATIVE DES FLAGELLÉS DU COBAYE. Léon Morénas. Annales de l'Université de Lyon. 3^e serie, Médecine. Fasc. 1. Paper. Pp. 234, with 12 illustrations. Price 60 francs. Paris: Masson & Cie, 1938.

SURGICAL PATHOLOGY OF THE DISEASES OF THE MOUTH AND JAWS. Arthur E. Hertzler, M.D., Surgeon to the Agnes Hertzler Memorial Hospital, Halstead, Kan., and Professor of Surgery, University of Kansas. Cloth. Pp. 248, with 206 illustrations. Price \$5. Philadelphia: J. P. Lippincott Company, 1938.

ANIMAL PATHOLOGY. Russell A. Runnells, D.V.M., M.S., Associate Professor of Veterinary Pathology, Iowa State College. Cloth. Pp. 464, with 127 illustrations. Price \$6. Ames, Iowa: Collegiate Press, Inc., 1938.

ANNUAL REPORT OF THE SURGEON GENERAL OF THE PUBLIC HEALTH SERVICE OF THE UNITED STATES FOR THE FISCAL YEAR 1938. Cloth. Pp. 184. Price 60 cents. Washington, D. C.: U. S. Government Printing Office, 1938.

THE PATIENT IS THE UNIT OF PRACTICE. Duane Willard Propst, A.B., B.S., M.D., Assistant Professor of Medicine, University of Illinois College of Medicine. Cloth. Pp. 219. Price \$3.50. Springfield, Ill.: Charles C. Thomas, Publisher, 1939.

MEASLES. REPORT OF THE MEDICAL OFFICER OF HEALTH AND SCHOOL MEDICAL OFFICER ON THE MEASLES EPIDEMIC 1935-1936. Paper. Pp. 94. Price 1 shilling. London: London County Council, 1938.

ARBEITEN AUS DEM SERO-BAKTERIOLOGISCHEN INSTITUT DER UNIVERSITÄT HELSINKI. Herausgegeben von Prof. Dr. Osw. Streng. Vol. 10 (1937-1938). Various pagination. Helsinki, 1938.

NATURE OF VEGETATIONS OF BACTERIAL ENDOCARDITIS

ARTHUR C. ALLEN, M.D.

NEW YORK

The history of the investigations of endocardial vegetations has been reviewed by Perry¹ in his monograph and by Gross in his series of papers on rheumatic endocarditis. It suffices to mention here that in the early part of the nineteenth century, Corvisart² thought that the vegetations of bacterial endocarditis represented syphilis, because of their resemblance to venereal warts. This was followed by the belief that they were made of "coagulable lymph" (e. g., Cayley³). However, it soon became apparent that the lesions resembled thrombi inasmuch as they were thought to be composed of platelets, fibrin and blood cells.

The latter time-honored concept has become deeply rooted in textbooks and in current literature. It is now generally assumed without question that the bulk of each of these characteristic shaggy vegetations is simply a thrombotic mass of platelets with enmeshed fibrin, red blood cells and leukocytes, often capped by clumps of bacteria. This thrombus is thought to be deposited from the blood flowing over the inflamed endocardial surface of the valve (MacCallum⁴; Boyd⁵). It is agreed that frequently areas of necrosis may be seen within the thrombi and that subsequently organization and calcification may take place.

For purposes of orientation in this discussion, it may be stated that a typical vegetation of acute or subacute bacterial endocarditis is com-

From the Department of Pathology, Cook County Hospital; Dr. R. H. Jaffé, director (deceased).

1. Perry, C. B.: *Bacterial Endocarditis*, Bristol, John Wright & Sons, Ltd., 1935.

2. Corvisart, J. N.: *A Treatise on the Diseases and Organic Lesions of the Heart and Great Vessels*, translated by C. H. Hebb, London, Underwood & Blacks, 1813.

3. Cayley, W.: *M. Times & Gaz.* 2:509, 1877.

4. MacCallum, W. G.: *A Text-Book of Pathology*, ed. 6, Philadelphia, W. B. Saunders Company, 1937, pp. 240-245.

5. Boyd, W.: *A Text-Book of Pathology*, ed. 1, Philadelphia, Lea & Febiger, 1932, p. 148.

monly regarded as being composed of three rather poorly defined zones (MacCallum⁴; Hadfield and Garrod⁶):

Zone 1. A proximal layer of fibrin, platelets, red blood cells and leukocytes with more or less necrosis.

Zone 2. A layer of bacteria.

Zone 3. An outermost layer of fibrin in which red blood cells and a sprinkling of leukocytes are enmeshed.

CURRENT VIEW

The existence in general of these zones is not denied. Differences arise, however, in the interpretation of the zone underlying the bacteria. This, zone 1, exclusive of the bacterial layer, usually makes up the bulk of the vegetation, so that an accurate knowledge of its composition is the sine qua non of a true concept of the histogenesis of the lesion. Those who assume a thrombotic origin consider this zone to be composed of platelets, fibrin and blood cells which have been deposited from the blood stream within the cardiac chambers and which have subsequently undergone more or less necrosis.

OPPOSING VIEW

However, papers have appeared from time to time in the German literature suggesting that these lesions are nonthrombotic (Baldasari⁷). More recently, Jaffé⁸ reemphasized the view that the vegetations are not thrombi deposited on the valve but are derived from the tissue of the valve itself. Nevertheless, the thrombotic concept persists. One is unable to find in the literature actual studies of bacterial vegetations which show irrefutably that they are or are not of valvular origin. Vegetations have, of course, been previously sectioned and carefully studied, but studies of *differentially stained* sections with this issue in mind appear wanting except in rare instances.

MATERIALS AND METHODS

Vegetations from 5 hearts with acute and 19 with subacute bacterial endocarditis were sectioned and stained with hematoxylin-eosin, Weigert's elastica Van Gieson, Mallory's phosphotungstic acid-hematoxylin, Mallory's aniline blue and Foot's reticulum stains. Five of the lesions were sectioned serially. The vegetations varied in size from 0.5 mm. to 17 mm. in their greatest dimension. Fifteen (two acute and thirteen subacute) were superimposed on fibroplastic valvular deformities.

6. Hadfield, G., and Garrod, L. P.: *Recent Advances in Pathology*, Philadelphia, P. Blakiston's Son & Co., 1934.

7. Baldasari, V.: *Centralbl. f. allg. Path. u. path. Anat.* **20**:97, 1909.

8. Jaffé, R. H.: *Virchows Arch. f. path. Anat.* **287**:379, 1932.

OBSERVATIONS

Elastic and Collagenous Tissue.—With the simple hematoxylin-eosin stain, it was found impossible in the great majority of instances to be certain of the nature of the necrotic zone 1. The morphologic resemblance to simple platelet thrombi is certainly undeniable. With differential stains, however, isolated patches and strands of elastic and collagenous fibers were plainly visible within these "platelet" masses in many sections (figs. 1 and 2). Some were swollen and merged at one end with clear-cut wavy fibers, and on the other, with necrotic hazy shreds, finally shading off into an amorphous homogeneous granular material.

Frequently, solitary clumps of adult elastic and collagenous fibers, sometimes a single anuclear strand, were seen in a bed of necrotic debris near the periphery of the vegetation. The ends of these fibers were often frayed and ragged as if fragmented by the intense, widespread edema and necrobiosis and then forcibly pushed distalward from their original location (figs. 3 and 4). These separated foci of fibers stained quite like those elsewhere, e. g., the intact chordae tendineae.

Elastic or collagenous tissue was found in all but two of the vegetations. These were from hearts showing acute endocarditis and were composed simply of unidentifiable necrotic tissue and huge masses of bacteria. But even in these, collagenous fibers were seen fanning out into the vegetations from their bases. The edges of these fibers did not taper smoothly, as one might expect of granulation tissue, but were irregular and fragmented; so that one can hardly escape the impression that their distal ends originally extended farther into the vegetation—as in other instances—but were destroyed by the necrotic process.

The question naturally arises: May not these elastic or collagenous fibers be a part of the current reparative fibroplastic proliferation within the "thrombotic" mass? However, this seems hardly likely, since these same adult ragged strands of collagenous tissue may be found isolated near the periphery of acute vegetations (fig. 1) in which there has not been sufficient time for the formation of mature tissue of this character.⁹ Furthermore, their anuclear nature and their complete separation from, or lack of continuity with, a nidus of fibroplastic proliferation, which nidus may be entirely absent if the infection is sufficiently virulent and recent, appear to prove that these fibers antedate the vegetation. The source of these fibers must therefore be the underlying fibroplastic deformity or—in the minority of cases—the fibroelastic and collagenous tissue of the normal valve. In other words, they evidence a destructive rather than a reparative process.

9. Maximow, A.: Beitr. z. path. Anat. u. z. allg. Path. **38**:301, 1905; Physiol. Rev. **4**:533, 1924; Text-Book of Histology, Philadelphia, W. B. Saunders Company, 1930, p. 141.

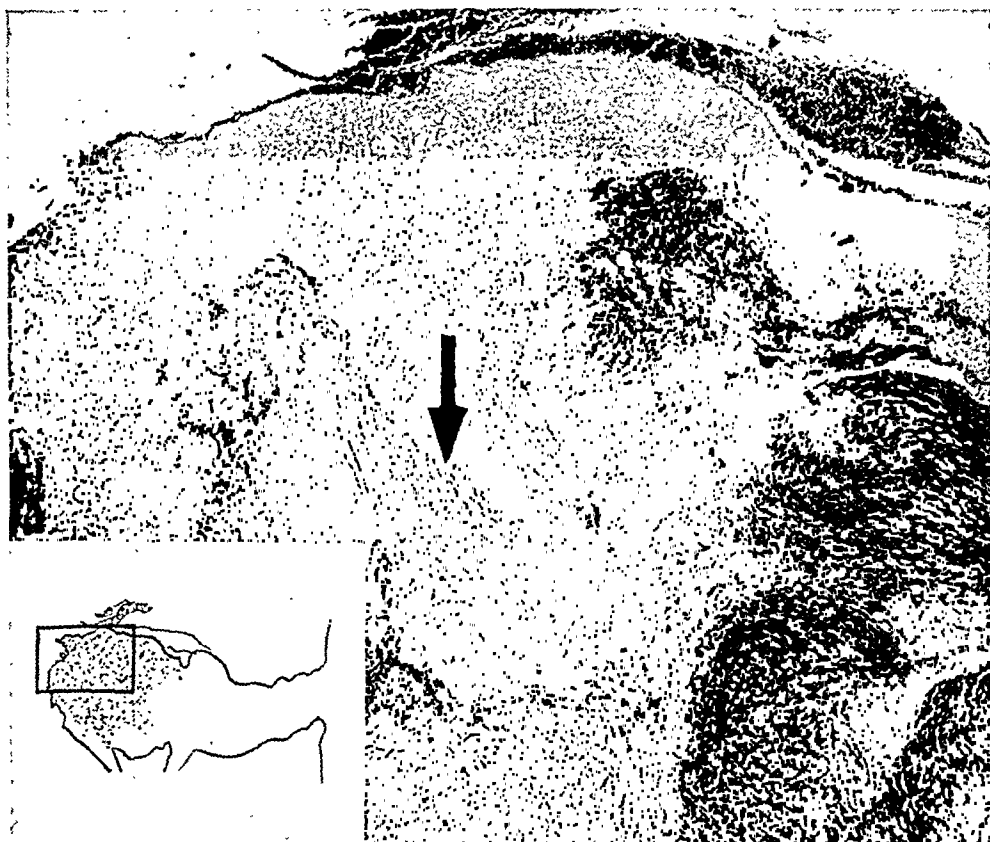


Fig. 1.—Vegetation of subacute endocarditis due to infection with *Str. viridans*, from mitral valve. There had been previous rheumatic deformity. This is a good example of crumbled, displaced, partially degenerated elastic strands and clumps squarely within a typical shaggy vegetation, ordinarily considered a simple thrombotic mass for the most part. Note that some of the fibers show partial loss of affinity for the elastica stain so as to resemble fibrin superficially (arrow). (Weigert's elastica Van Gieson stain; low power magnification.)

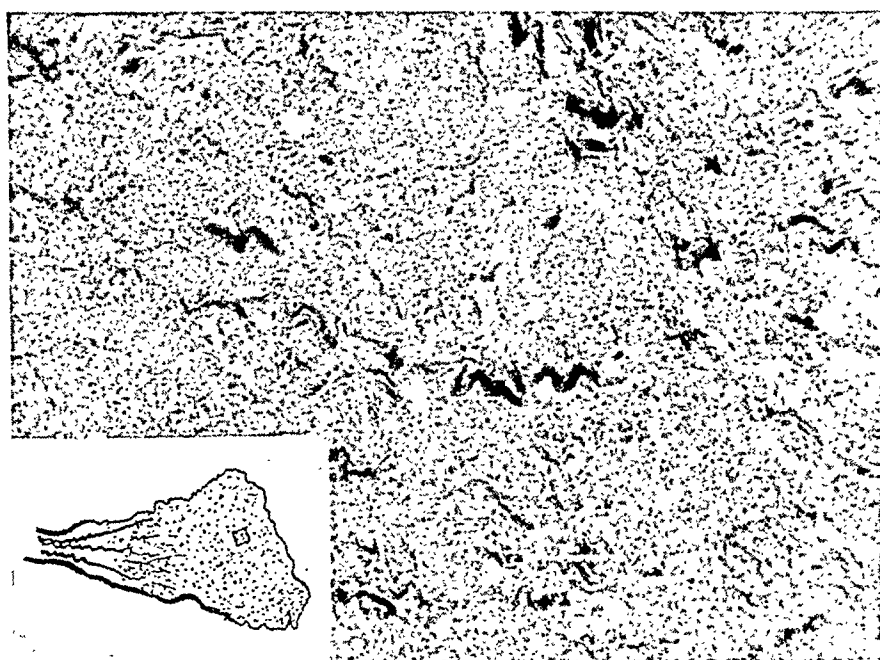


Fig. 2.—Acute endocarditis of the tricuspid valve due to infection with a hemolytic strain of *Staphylococcus aureus* (condition of five days' duration, clinically). There had been no previous apparent rheumatic involvement. Note the haphazard distribution of apparently forcibly separated, fragmented, disrupted collagenous fibers in various states of preservation, lying in a bed of disintegrating blood cells and granular precipitate. This illustrates the destructive factor in the origin of vegetations from valvular components which bulge outward into the chamber. (Van Gieson stain; high power magnification.)

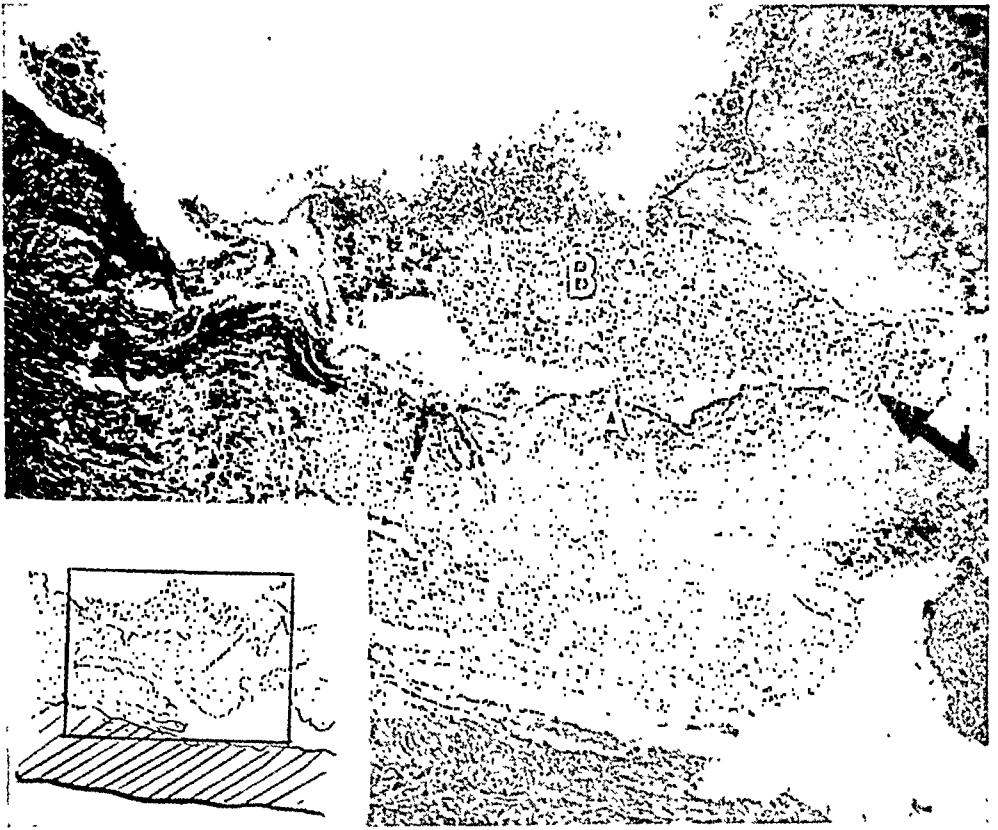


Fig. 3.—Vegetation of subacute endocarditis due to infection with *Str. viridans*, from the wall of the left auricle. Note that the “thrombotic mass” is overlaid in part by disrupted elastic fibers (*A*). Single strands (arrow) are seen as if actually forced through the debris, their distal ends lying free in the terminally deposited, well preserved blood (*B*). (Weigert’s elastica Van Gieson stain; low power magnification.)



Fig. 4.—Vegetation of subacute endocarditis due to infection with *Str. viridans*, from mitral valve. Note the disrupted and distorted black elastic fibers at the periphery of the vegetation embedded in the necrobiotic debris and exudate. Also note the separation of elastic fibers as if forced apart by such material. (Weigert’s elastica Van Gieson stain; low power magnification.)

These elastic and collagenous fibers may be easily and unequivocally differentiated from granulation tissue (and fibrin) by morphology, by their relationship to neighboring tissue, by their staining characteristics and by the duration of the lesions.⁹ This was further confirmed by a study of sterile and infected thrombi as controls. Therefore, it may be concluded that these isolated adult fibers at the periphery of the vegetation appear to be definite evidence in favor of the view that these lesions arise from valvular tissue and to be distinctly contrary to the current

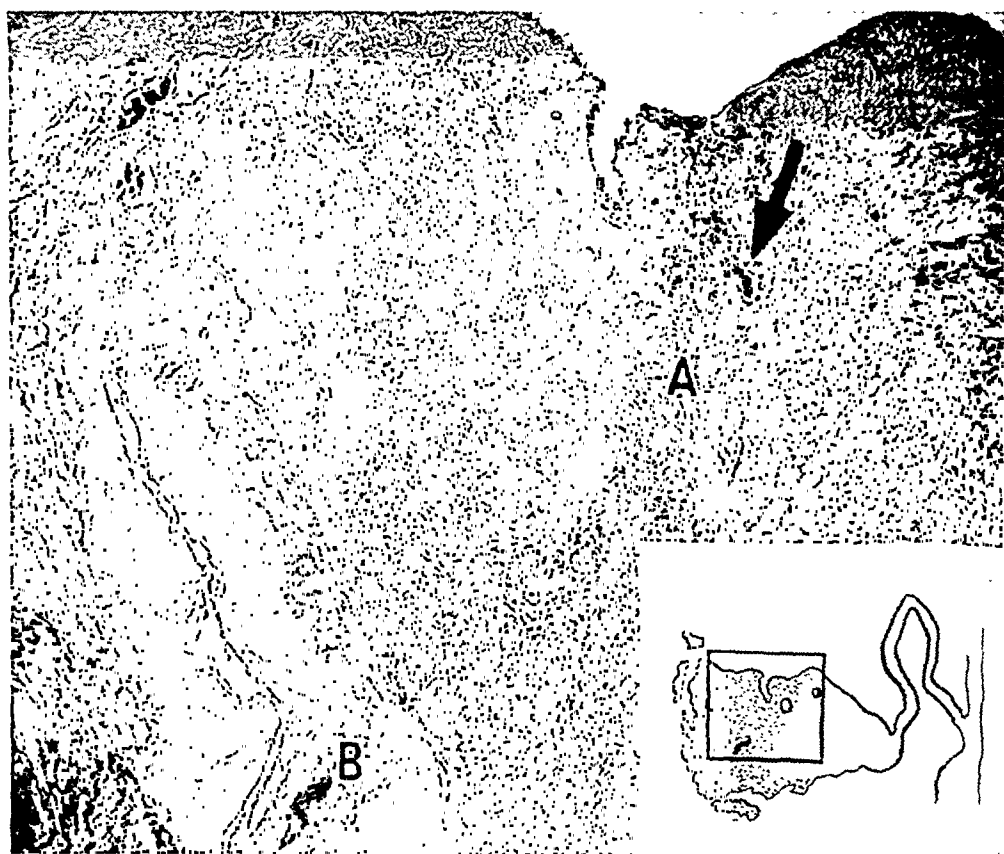


Fig. 5.—Vegetation of subacute endocarditis due to infection with *Str. viridans*, from mitral valve. There had been previous rheumatic deformity. Note so-called thrombotic mass extending from the left margin to A, consisting of necrobiotic valvular debris, fibrin, polymorphonuclear leukocytes, red blood cells, coagulated granular precipitate and displaced clumps and strands of elastic fibers. Note the proximity of blood vessels from the old fibroblastic deformity (arrow) to the vegetation. Such vessels, necrosed by the advancing ulcerative process, appear to be the source of the original and of additional blood elements within the vegetation. This is partly the reason for the absence of any characteristic arrangement such as one may find in vascular thrombi. Note the isolated clumps of elastic fibers within the fibrin mass (B). (Weigert's elastica Van Gieson stain; low power magnification.)

concept, according to which the material is deposited from the blood within the chambers.

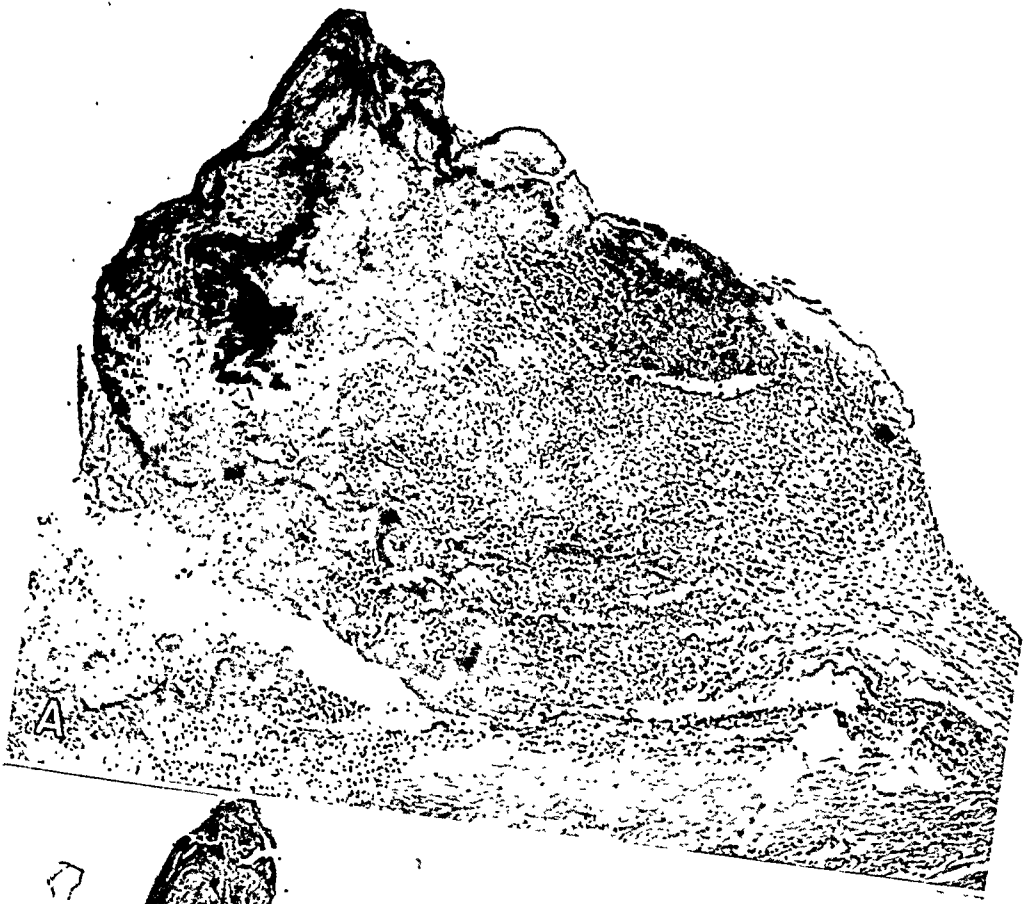


Fig. 6.—Vegetation of subacute endocarditis due to infection with *Streptococcus viridans*, from the outflow tract of the left ventricle. Note the divergence of the vegetation from the endocardium. In the section stained by Weigert's elastica Van Gieson stain (*B*), a strand of isolated elastic fibers (arrow) is seen within the "thrombotic mass" as if forced upward by the underlying necrobiotic debris and exudate. The section stained with hematoxylin and eosin (*A*) gives no hint as to the presence of such displaced fibers.

Vascularity.—Capillarization was seen directly within the body of all vegetations except 2, which were described in the foregoing text. The latter may be accounted for by the overwhelming necrosis, which one may conclude destroyed the elastic and collagenous fibers as well as the capillaries which may have been present.

This observation requires further discussion. Inasmuch as Gross stated that normal valves are not vascularized, what is the source of these capillaries? In the first place, 15 of this series were superimposed on obvious preexisting valvular deformities. (This is approximately the ratio for superimposition of bacterial endocarditis found by Libman,¹⁰ Thayer,¹¹ Blumer¹² and others.) It is, of course, well known that rheumatic valvular lesions are vascularized (Gross¹³). In such cases, then, it is reasonable to believe that the red and white blood cells, coagulable plasma (fibrin) and "platelets" come from these vessels and their offshoots.

In the remaining vegetations there was no apparent underlying fibroplastic deformity as far as one was able to judge from necrotic lesions. What, then, was the source of these vessels, since normal valves, as mentioned, are perhaps not vascularized? In answer to this, it may be stated that vascularization of a valve may be part and parcel of the inflammatory response to bacteria, just as it may be elsewhere. It is not as if the vegetation were suddenly capped onto the valve. Even in acute cases (duration of six weeks or less by general agreement) there is sufficient time for thin-walled capillaries to form. Proof for this statement, in addition to the data included herewith, is furnished in valves from patients suffering the first attack of acute rheumatic fever—of six weeks' duration or less. In these Gross and Friedberg found distinct hypercapillarization throughout the valve leaflet. It is interesting in connection with this question of vascularity to recall that Von Glahn and Pappenheimer¹⁴ expressed the belief that acute rheumatic endocarditis precedes all bacterial endocarditides. If this is so, the vascularity might be accounted for by this rheumatic process. Furthermore, Clawson¹⁵ and also Bell and Hartzell¹⁶ stated that there is diffuse inflammation of the leaflet in the early stages of bacterial endocarditis. The possibility of

10. Libman, E.: J. A. M. A. **80**:813, 1923.

11. Thayer, W. S.: Johns Hopkins Hosp. Rep. **22**:1, 1926.

12. Blumer, G.: Medicine **2**:105, 1923.

13. Gross, L., and Friedberg, C. K.: Am. J. Path. **12**:855, 1936.

14. Von Glahn, W. C., and Pappenheimer, A. M.: Arch. Int. Med. **55**:173, 1935.

15. Clawson, B. J.: Arch. Int. Med. **33**:157, 1924.

16. Clawson, B. J.; Bell, E. T., and Hartzell, T. B.: Am. J. Path. **2**:193, 1926.

the formation of superficial capillary sprouts from the endothelium of the surface of the valves must also be definitely reckoned with. In short, there is abundant evidence demonstrating that valves with bacterial endocarditis become vascularized even when there has been no apparent gross antecedent fibroplastic deformity. It is accordingly maintained that these vessels are the source of the red and white blood cells, coagulable plasma (fibrin) and "platelets" that may be found beneath the layer of bacteria. Furthermore, inasmuch as indisputably valvular

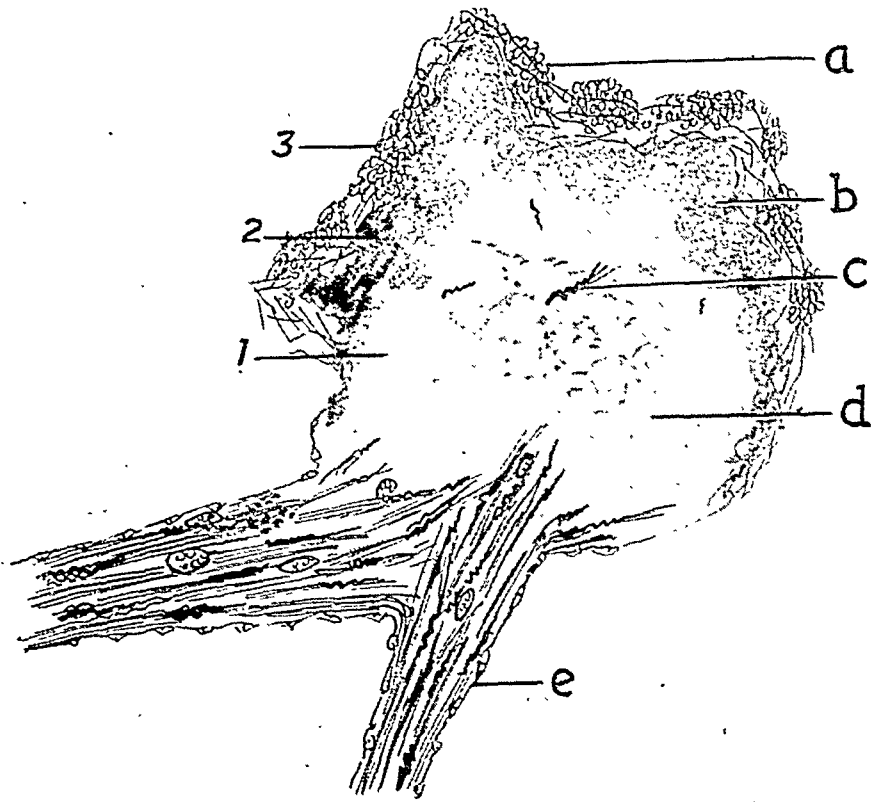


Fig. 7.—Diagrammatic representation of a typical bacterial vegetation. 1, 2 and 3 indicate zones 1, 2 and 3, respectively; a indicates a superficial thrombus from blood within cardiac chambers; b, bacteria; c, collagenous fibers; d, necrotic collagenous fibers, blood cells and fibrin; v, chordae tendineae.

elastic and collagenous tissue is found overlying these blood elements, one may assume from this fact that these elements are not deposited in thrombotic fashion from the blood stream flowing over the valve but are derived from the various constituents of the inflamed or deformed valve.

"Platelet Mass."—The necrotic zone 1 beneath the bacteria is generally considered to be composed of a platelet mass to a great extent

(Gross and Fried;^{16a} MacCallum;⁴ Kaufman;¹⁷ Clawson,¹⁵ and others). However, it must be emphasized that this is predominantly a necrotic zone. Therefore, it does not seem justifiable to describe a more or less homogeneous granular material as a mass of platelets in the face of the very palpable possibility that that material may be the residue of necrobiosis and therefore truly unidentifiable. However, one may perhaps judge the nature of this material from the character of the surrounding tissue. As was mentioned, elastic and collagenous fibers were frequently seen radiating and finally disappearing into the necrotic mass to appear again in scattered random areas (figs. 3 and 4). Between these separated fibers, red and white blood cells were seen also merging with this homogeneous substance. Therefore, it seems more reasonable to conclude that this granular material represents fibrous tissue and blood elements which have undergone necrobiosis rather than simply essentially a "platelet mass," as it is currently regarded. This tends further to refute the current concept of the nature of the bacterial vegetation.

The remainder of the vegetation consists distally of a rim of bacteria and a layer of simple thrombotic material, apparently deposited from the blood as it flows over the valve. This last zone does not appear to be an intrinsic part of the lesion, however. One is often impressed with the good state of preservation of this layer—as if it constituted a more or less terminal deposit.

Bacterial Layer.—The frequency with which a band of bacteria is found near the periphery of the vegetation, both in human and experimental endocarditides (Rosenow¹⁸), is not reconcilable with the concept that the lesion is a thrombus (Jaffé⁸) deposited from the blood of the chamber. If this concept were a fact, one would expect the thrombus to overlie the organisms, inasmuch as they are, of course, assumed to precede the vegetation. Yet, the major portion of the vegetation generally underlies the bacteria. In defense, Perry¹ maintained that the organisms have originally been at the base but have lost their affinity for stains. If this is true, one wonders why there is no stratification showing the transition from the dead bacteria proximally to more viable ones peripherally instead of the abrupt rim so often seen at the surface.

In concluding, it is believed that the evidence that the bacterial vegetation is not a simple thrombus deposited from blood flowing over the valve concerns an issue greater than that of the mere morphologic nicety. For example, on this point may rest supporting evidence for an important, well recognized concept, namely, that tissue immunity in the broad con-

16a. Gross, L., and Fried, B. M.: *Am. J. Path.* **13**:769, 1937.

17. Kaufman, E.: *Pathology for Students and Practitioners*, translated by S. P. Reimann, Philadelphia, P. Blakiston's Son & Co., 1929, vol. 1, pp. 25-37.

18. Rosenow, E. C.: *J. Infect. Dis.* **7**:411, 1910; **11**:210, 1912.

notation of allergy¹⁹ is a factor in the production of bacterial endocarditis. If one is permitted the luxury of speculatively integrating morphologic observations with vital phenomena, one is led to believe that the marked necrosis and edema, with ripping and separation of originally closely packed elastic and collagenous fibers, are strongly reminiscent of a response to an altered local tissue reactivity.

SUMMARY

The structure of the vegetations in 24 cases of acute and subacute bacterial endocarditis was studied with the aid of differential connective tissue stains.

In agreement with current descriptions, the bulk of each of these vegetations was found generally to consist of a necrotic zone beneath a layer of bacteria.

Isolated clumps, fragmented strands and segmented bits of typical adult elastic and collagenous tissue, easily distinguished from granulation tissue or fibrin, were found scattered through this necrotic zone in both acute and subacute vegetations. *These fibers are considered evidence of a destructive rather than of a reparative process.*

The question as to whether or not this necrotic zone is really essentially a platelet mass, in accordance with the current concept, is discussed on the basis of morphologic observations.

It is concluded, contrary to the long established current concept, that the bulk of a vegetation of acute or of subacute bacterial endocarditis is derived from components of inflamed and fibroplastically deformed valves and is not derived from blood flowing over the valves.

1 East 100th Street, New York City.

19. Semsroth, K., and Koch, R.: Arch. Path. **10**:867, 1930. Wadsworth, A. B.: J. M. Research **39**:279, 1919. Derick, C. L., and Swift, H. F.: Proc. Soc. Exper. Biol. & Med. **25**:222, 1922. Kinsella, R. A., and Sherburne, E. C.: J. A. M. A. **80**:1643, 1923. Wright, A. D.: J. Path. & Bact. **29**:5, 1926. Swift, H. F.: Am. Heart J. **3**:629, 1928.

PROGRESSIVE ALCOHOLIC CIRRHOSIS

A CLINICAL AND PATHOLOGIC STUDY OF SIXTY-EIGHT CASES

ERNEST M. HALL, M.D.

AND

WENDELL A. MORGAN, M.D.

LOS ANGELES

Interest in Laënnec's cirrhosis continues, owing, no doubt, to the importance of the condition as a disease and to the fact that the etiologic factors are still in question. The old controversy as to the role of alcohol in cirrhosis continues. Attempts to produce cirrhosis in animals by the use of alcohol have almost invariably failed. Physiologists and experimental pathologists are, on the whole, opposed to the idea that alcohol is a direct cause of cirrhosis. Mallory¹ stated that after thirty-six years of experimentation on alcoholic cirrhosis he, like other investigators, had ruled out ethyl alcohol as the cause. Boles and Clark,² from a study of the records of 4,000 autopsies made at the Philadelphia General Hospital from 1933 to 1935, concluded that alcohol cannot be regarded as a specific factor in the causation of cirrhosis. They suggested abandonment of the term "alcoholic cirrhosis."

It is generally agreed that ethyl alcohol is not a strong hepatic poison like arsenic or phosphorus, nor is it so active in producing necrosis of the liver as chloroform and carbon tetrachloride. Nevertheless, when alcohol is injected in doses of 0.1 cc. directly into the portal circulation, it produces localized necroses of the liver, as shown by Ogata³ and more recently by Cameron, Karunaratne and Thomas.⁴ Similar changes, but with massive infarct-like necroses, are produced by the injection of chloroform (Whipple and Sperry;⁵ Schultz, Hall and Baker⁶) and by the injection of carbon tetrachloride (Schultz and Marx;⁷ Cameron,

From the departments of pathology of the Los Angeles County Hospital and the School of Medicine of the University of Southern California.

1. Mallory, F. B.: *Am. J. Path.* **9**:557, 1933.

2. Boles, R. S., and Clark, J. H.: *J. A. M. A.* **107**:1200, 1936.

3. Ogata, S.: *J. M. Research* **40**:103, 1919.

4. Cameron, G. R.; Karunaratne, W. A. E., and Thomas, J. C.: *J. Path. & Bact.* **44**:297, 1937.

5. Whipple, G. H., and Sperry, J. A.: *Bull. Johns Hopkins Hosp.* **20**:278, 1909.

6. Schultz, E. W.; Hall, E. M., and Baker, H. V.: *J. M. Research* **44**:207, 1923.

7. Schultz, E. W., and Marx, A.: *Am. J. Trop. Med.* **4**:469, 1924.

Karunaratne and Thomas ⁴). Cameron and his associates studied these changes carefully and expressed the belief that such lesions are due to a direct toxic action of the poison on the liver cells. They have proposed the term "toxic infarction," which is an apt one.

Granted that alcohol, chloroform and carbon tetrachloride produce hepatic necrosis under the conditions outlined, it does not follow necessarily that cirrhosis results from the healing of such lesions. As already noted, true Laënnec's cirrhosis had not resulted from experiments in which alcohol was given to animals, although some periportal proliferation of fibrous tissue has been reported by several investigators. Whipple and Sperry ⁵ failed to find cirrhosis following prolonged chloroform anesthesia (two to three hours) in dogs although central necrosis of the hepatic lobules inevitably resulted from the effects of the anesthetic. Several of the dogs died from acute hepatic necrosis within a period of two to seven days after their anesthesia. In the dogs that survived the administration of chloroform, complete restoration of the hepatic lobules occurred by regeneration of the liver cells.

Herter and Williams, ⁸ however, reported that definite cirrhosis occurred when dogs were anesthetized with chloroform three times per week. One dog received a total of eighteen, another a total of forty-nine, inhalations. The animals lived five and eight months, respectively. Data as regards carbon tetrachloride cirrhosis are still more convincing because of the work of Lamson and Wing, ⁹ Bollman and Mann ¹⁰ and Cameron and Karunaratne. ¹¹ The latter found well marked cirrhosis of a permanent type in rats that had received from twenty-eight to forty doses of 0.1 cc. of carbon tetrachloride at the rate of two a week. When only sixteen to twenty-one doses were given, the fibrosis was only temporary and usually disappeared within two to three weeks. Cameron and Karunaratne pointed out that cirrhosis results from injections of carbon tetrachloride only when the following conditions are fulfilled: 1. A dose greater than the minimal toxic dose for the liver must be used. 2. It must be administered either continuously or at short intervals over a prolonged period. 3. The intervals between doses must be sufficiently short to avoid complete repair of the damage produced by the preceding dose. Herter and Williams ⁸ seem to have fulfilled these requirements in producing chloroform cirrhosis in dogs. Von Glahn, Flinn and Keim ¹² showed recently in rabbits fed lead,

8. Herter, C. A., and Williams, W. R.: *Proc. Soc. Exper. Biol. & Med.* **3**: 23, 1905.

9. Lamson, P. D., and Wing, R.: *J. Pharmacol. & Exper. Therap.* **29**: 191, 1926.

10. Bollman, J. L., and Mann, F. C.: *Ann. Int. Med.* **9**:617, 1935.

11. Cameron, G. R., and Karunaratne, W. A. E.: *J. Path. & Bact.* **42**:1, 1936.

12. Von Glahn, W. C.; Flinn, F. B., and Keim, W. F.: *Arch. Path.* **25**: 488, 1938.

copper and sodium arsenates an incidence of cirrhosis above 90 per cent. The animals received 5.6 to 12 mg. of one of the aforementioned salts daily for periods ranging from one hundred to two hundred days. Here again the principle of many, closely timed injuries to the liver over a considerable period resulted in cirrhosis in a very high percentage of the animals.

Alcohol, which is less toxic for the animal liver than either chloroform or carbon tetrachloride, probably has to be administered in correspondingly larger doses over a long period in order to produce severe changes in the liver. More recently evidence has been accumulating which indicates that for man, at least, the body and more especially the liver must be in a state of altered metabolism before cirrhosis will result from heavy drinking of alcoholic liquor. That abnormal changes take place in metabolism, more specifically in the utilization of carbohydrates, fats and proteins, in the patient who drinks a pint of whisky per day for twenty or twenty-five years is well known. That the liver suffers from alcoholic overindulgence is evident from the great increase in fat, an increase generally accepted clinically and proved experimentally to be due to alcohol (Ruge;¹³ Friedenwald;¹⁴ Fahr¹⁵). The liver of the consumer of alcohol is also very low in glycogen content (LeCount and Singer¹⁶). It is probably not only the toxicity of alcohol itself but also the increased susceptibility of the already damaged cells of the liver to a mild hepatic poison under conditions of changed carbohydrate metabolism, lowered intake of food and deficiency in certain vitamins that makes possible the changes seen in cirrhosis. Furthermore, there is some evidence for an individual idiosyncrasy toward alcohol, as there is toward many drugs, since cirrhosis develops in only 5 to 6 per cent of persons addicted to the use of alcoholic beverages.

There can be no doubt that portal cirrhosis is produced by a considerable number of agents (Moon¹⁷). Studies of Laënnec's cirrhosis in the chronic form ordinarily show an alcoholic history in but 25 (Evans and Gray¹⁸) to 35 per cent (Boles and Clark²) of the cases. Does this necessarily mean that there is no true alcoholic cirrhosis? Or does it indicate simply that investigators have failed to recognize the alcoholic group because toxic agents other than alcohol are capable of producing a portal type of cirrhosis which in the late stages is not distinguishable from the alcoholic variety? We believe that the latter is the case. We maintain that the term "alcoholic cirrhosis" is fully justi-

13. Ruge, P.: *Virchows Arch. f. path. Anat.* **49**:252, 1870.

14. Friedenwald, J.: *J. A. M. A.* **45**:780, 1905.

15. Fahr, T.: *Verhandl. d. deutsch. path. Gesellsch.* **13**:163, 1909.

16. LeCount, E. R., and Singer, H. A.: *Arch. Path.* **1**:84, 1926.

17. Moon, V. H.: *Arch. Path.* **18**:381, 1934.

18. Evans, N., and Gray, P. A.: *J. A. M. A.* **110**:1159, 1938.

fiable. In the following pages we shall analyze a group of cases in which we believe the true picture of alcoholic cirrhosis in the early, progressive stages was presented.

Evans and Gray¹⁸ recently published a study of 217 cases of Laënnec's cirrhosis which were observed among 17,000 autopsies at the Los Angeles County Hospital. They were especially interested in the relation of alcohol to cirrhosis and showed a definite increase in the incidence of this disease beginning in 1932 soon after the repeal of the national prohibition law. Our cases are taken from the same group of autopsies as were the cases of Evans and Gray; however, some 2,000 autopsies from May 1, 1937, to May 1, 1938, have been included in our series that were not in theirs. None of our cases goes back beyond autopsy 7,000, since sufficient data could not be assembled from the earlier cases on which to separate them clearly into our group. The two subgroups overlap sufficiently, however, for us to use the larger series of 217 cases studied by Evans and Gray for the purposes of comparison.

Our 68 cases were selected as instances of the progressive, or active, type of alcoholic cirrhosis as described by Hall and Ophüls.¹⁹ This condition may be conveniently designated as subacute alcoholic cirrhosis. The liver is enlarged, the weight ranging from 2,000 to 5,000 Gm., and is usually but not always fatty. Eighty-five per cent of the patients were consumers of alcohol in some form. Fifty-one patients, or 75 per cent, were shown to have chronic alcoholism; i. e., they were "heavy drinkers."

Other characteristics of the liver in subacute alcoholic cirrhosis are as follows: The surfaces of the organ are smooth or finely granular rather than the hobnail type seen in chronic cirrhosis. The fibrosis is generally less severe than in the chronic type, while cellular or relatively cellular connective tissue proliferation is found in the portal areas. Necrosis of liver cells is still present, in many cases being quite marked. Fibroblastic cells may usually be seen proliferating about the dead hepatic cells. Polymorphonuclear leukocytes are frequently present along with the lymphocytic cells which infiltrate the periportal connective tissue.

This group of patients were, then, quite largely suffering from alcoholism. The livers were mostly of the large, pale yellow fatty type, with the connective tissue actively proliferating about disintegrating hepatic cells. The picture was that of early or subacute alcoholic cirrhosis.

ANALYSIS OF CLINICAL DATA

These 68 cases were selected from among autopsies 7,000 to 20,000 at the Los Angeles County Hospital. The 217 cases selected by Evans and Gray include some of ours and many additional cases of alcoholic cirrhosis of the chronic hobnail type.

19. Hall, E. M., and Ophüls, W.: *Am. J. Path.* 1:477, 1925.

Race.—Caucasians predominate with 57 patients, or 85 per cent. Ethiopians are next most numerous with 6, or 8.8 per cent, and then Mexicans with 5, or 7.35 per cent.

Sex.—As would be expected, males are found to exceed females nearly 2 to 1, the actual numbers being 44 and 24. The average age at death for the whole group is 46.8 years, as contrasted with 60 years in the larger group (Evans and Gray). Figure 1 is a graph comparing our series with that of Evans and Gray in respect to the ages at death, by decades.

The youngest patient in our group was 24 years old at the time of death; the oldest was 75. Only 4 patients died in each of the corresponding decades. It is evident from figure 1 that in cases of subacute alcoholic cirrhosis death occurs mainly between the ages of 35 and 55 while in cases of chronic cirrhosis it occurs about ten years later. In the group with the chronic condition the "ages of death" also show wider

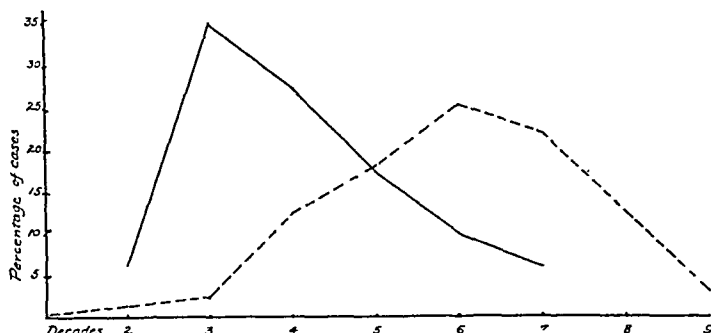


Fig. 1.—Graph comparing the distribution of ages at death, in decades, in the authors' cases (solid line) with that in the cases of Evans and Gray (broken line).

distribution. Heavy drinking is not frequently encountered before the twentieth or after the seventieth year. We cannot agree with Connor²⁰ in his statement that cirrhosis usually develops between the fifth and sixth decades. In the cases in the Los Angeles County Hospital death intervenes at that time or earlier.

History of Alcoholism.—Fifty-one, or 75 per cent, of the 68 patients were sufferers from chronic alcoholism, most of them having imbibed large quantities of whisky, often as much as a pint (473 cc.) or even a quart (946 cc.) per day, over a period of five to twenty-five years. A few drank considerable quantities of wine, usually one to two quarts daily. Six patients were classified as "moderate drinkers" because of insufficient data. Three of these, we believe, had chronic alcoholism. In 10 cases no record as to alcoholic habits was made on the hospital

20. Connor, C. L.: Am. J. Path. 14:347. 1938.

charts. Ten of the 51 patients had one or another form of alcoholic psychosis. Seven had either pellagra or alcoholic neuritis.

Of the 10 patients with no record of having consumed alcoholic liquors, all but a single patient had severe acute infections. Most of them died within a few hours after entering the hospital. Three were in coma from the time of entry to the time of death. The only patient of this group who had no infection died of congestive heart failure. The histories of all but the last patient were necessarily deficient, and save in the 1 case the omission of data regarding the consumption of alcohol is probably justified.

The patients in this series were not selected because of their alcoholism but on the basis of the gross anatomic and histologic changes in their livers. Since 80 per cent of the whole group were consumers of alcoholic liquors, it seems logical to assume that 80 per cent of the patients whose alcoholic habits were not recorded were also consumers of alcoholic liquors. If the foregoing assumption is correct, 90 per cent of the entire group suffered from alcoholism.

Wassermann Reaction.—A positive Wassermann reaction was recorded in 12 cases, or 17.6 per cent. A negative Wassermann reaction was found in 39 cases. In 17 cases no data on this reaction were available. In only a single case in which the Wassermann reaction was strongly positive was there no record of alcoholism. Evans and Gray¹⁸ reported syphilis present in only 12 per cent of their cases. The question naturally arises as to whether or not syphilis plays a part in the production of alcoholic cirrhosis. Our series of cases suggests an etiologic relationship. The most extreme fibrosis encountered in this group was associated in a number of instances with a positive Wassermann reaction (fig. 2). Such a reaction was seen in 50 per cent of the cases in which the Wassermann reaction was positive and in only 20 per cent of the remainder. Schumacher²¹ emphasized the relation of syphilis to the production of a portal type of cirrhosis.

Jaundice, Ascites and Hemorrhage.—Jaundice was noted in 34 cases (50 per cent) of the series. In 24 cases the icteric index was 50 units or above; in some it was as high as 200 units. This figure (50 per cent) is considerably higher than that obtained for the mixed group (Evans and Gray), which was 29.5 per cent. It is to be expected that a group of patients suffering from a subacute type of cirrhosis in which hepatic necrosis and acute infection are prominent factors would exhibit a higher incidence of jaundice than would be found in a group suffering mainly from a more chronic form of hepatitis. Previous attacks of jaundice were recorded for 6 patients. These attacks occurred about six months

21. Schumacher, G. A.: Am. J. M. Sc. **194**:693, 1937.

to two years before the last entry into the hospital except in a single patient who reported that an attack had occurred fourteen years previously.

Ascites was present in 41 patients, or 60.3 per cent. It is interesting that Evans and Gray¹⁸ found ascites in 131 patients in their larger group of 217, or in 60.3 per cent. In 11 of our patients, ascites had been found previously—in 8 of these within six months to one year. One of the remaining 3 patients had had ascites two years before, and another, five

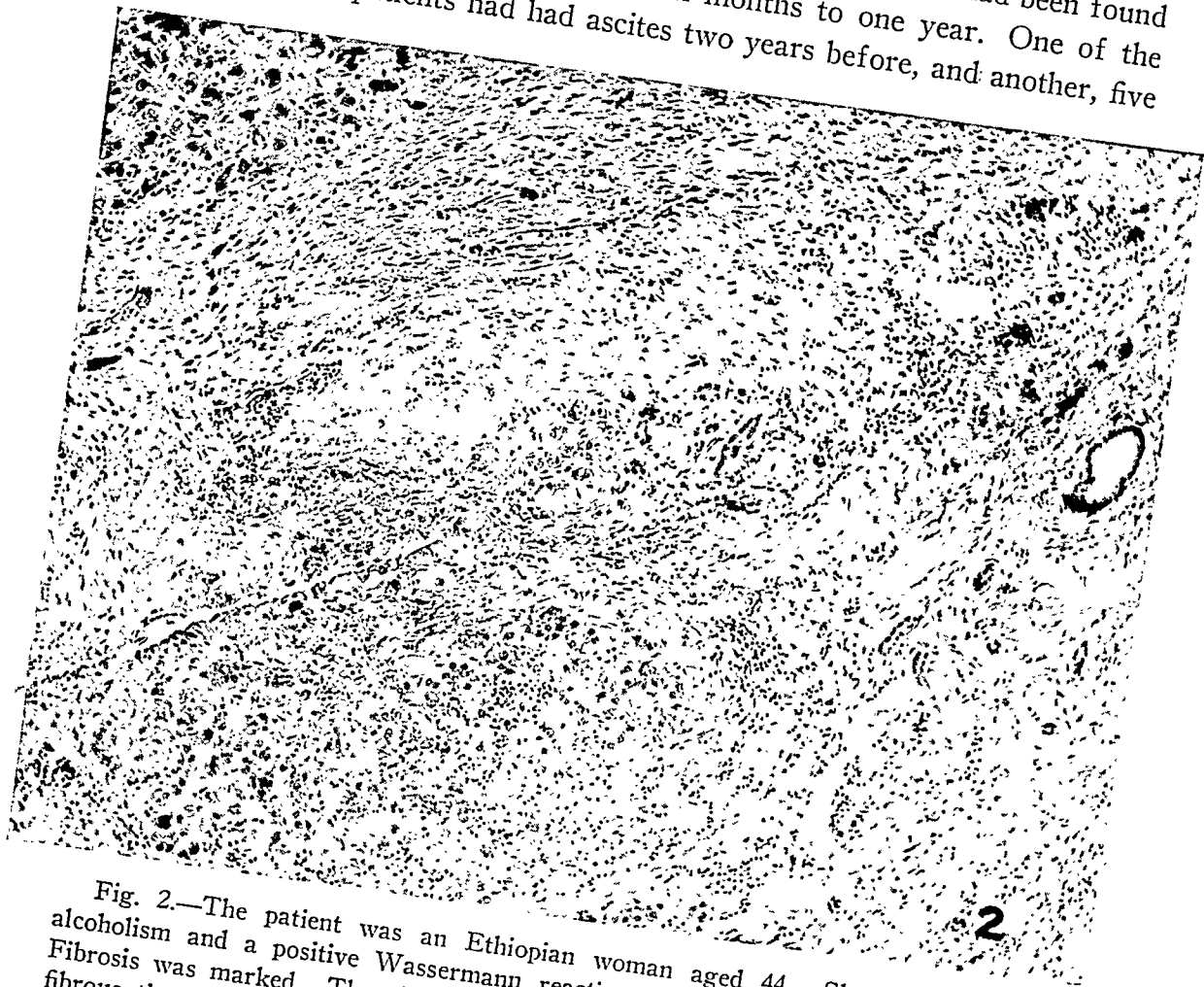


Fig. 2.—The patient was an Ethiopian woman aged 44. She had chronic alcoholism and a positive Wassermann reaction. The liver weighed 2,550 Gm. Fibrosis was marked. The photomicrograph (low power) shows dense bands of fibrous tissue, which are not confined to the periportal spaces. The pale areas are composed of a less dense connective tissue. Note the small groups of degenerating liver cells. Only a few new bile ducts are seen in this section. Hematoxylin and eosin; $\times 65$.

years previously, with enlargement of the liver; in the third abdominal swelling had been present for years. Varices of the esophagus with gastric hemorrhage were found in 24 (35.3 per cent). Varices were recorded in a few instances without

hemorrhage. Hemorrhage was fatal in 14 instances, or 20 per cent, which is a higher figure than that reported by Evans and Gray, 13.9 per cent.

Bloomfield²² recently emphasized the importance of previous attacks of jaundice or of hepatitis in cirrhosis. Eleven of the patients in our series had had earlier attacks of jaundice, and 14 gave evidence of previous hepatic disturbance. The data on these attacks are summarized in table 1.

The correct clinical diagnosis was made in 36 instances, or 52.9 per cent; the diagnosis was partially correct in 12 cases, or 17.6 per cent, and incorrect in 20 cases, or 29.4 per cent.

TABLE 1.—*Findings Suggesting Previous Attacks of Hepatitis*

Condition	Patients
Attacks of epigastric pain or of pain in the right upper quadrant of the abdomen (6 months to 7 years).....	6
Palpable or tender liver or both (6 months to 5 years).....	5
Hematemesis (with epigastric pain in 2 cases *), one 7 years previously.....	3
Fever and vomiting intermittently for 3 years.....	1
Indigestion, jaundice and ascites (6 months previously).....	1
	<hr/> 16

* Also listed under first item (total number of patients—14).

TABLE 2.—*Liver Weights*

Wt. Range, Gm.	Number
Below 2,000	5
2,000-3,000	39
3,000-4,000	15
4,000-5,000	6

ANALYSIS OF GROSS AND MICROSCOPIC CHANGES IN THE LIVER

Weight of the Liver.—The average weight of the liver in the 68 cases was 2,760 Gm. We have arbitrarily considered 2,000 Gm. the lower limit of hepatic weight for the subacute type of cirrhosis, though no hard and fast rule can be laid down. In a few of our cases, the weight of the liver fell below this figure. The smallest liver weighed 1,400 Gm.; 4 others ranged from 1,800 to 1,950 Gm. The largest liver in the series weighed 5,000 Gm.

The weight of the spleen was recorded in 66 of the 68 cases, the average being 360 Gm. This is approximately double the weight of the normal adult spleen. The increase in size was, no doubt to a large

22. Bloomfield, A. L.: Am. J. M. Sc. 195:429, 1938.

extent the result of chronic passive congestion. The organ was usually said to be firmer than normal. The exceptions were found in cases complicated by acute infection. Microscopically, there was diffuse fibrosis and usually there was reduction in the amount of lymphoid tissue.

In 40 instances, or 61.5 per cent, the surface of the liver was described as varying from smooth to finely granular, while in 25 instances, or 38 per cent, it was described as varying from roughly granular to nodular. The latter group, except for the enlargement of the organs, would appear to fall into the group of cases of the more chronic forms of Laënnec's cirrhosis. In 3 cases, the surface of the liver was not described.

Hepatic Fibrosis.—The relative amount of fibrosis in the various livers was estimated by microscopic examination and recorded as slight in 3 cases (4.4 per cent), moderate in 14 (20.6 per cent), marked in 32 (47 per cent) and extreme in 19 (or 28 per cent). All of the cases in which the Wassermann reaction was positive fell into one or the other of the last two groups. The amount of fibrous tissue proliferation in the general run of our cases seemed on the whole to be somewhat reduced in comparison with that seen in cases of the more chronic types of hepatitis. In distribution it was mainly periportal but not necessarily so. Of greater importance was the kind, or the maturity, of the connective tissue. Among the cases of subacute alcoholic cirrhosis we found 15 cases (22 per cent) in which it was classified as cellular; 11 (16.2 per cent) in which it was classified as moderately cellular; 37 (54.4 per cent) in which it was said to vary from moderately cellular to dense, and only 5 cases (7.4 per cent) in which it was designated as dense. Certainly the number of instances in which the connective tissue was cellular or moderately cellular (38 per cent) is greatly in excess of that noted for chronic atrophic cirrhosis (fig. 3). The largest group, 54.4 per cent, in spite of greater maturity, still showed evidence of fibroblastic proliferation, especially at the junction of fibrous tissue and liver cells and more especially about degenerating or necrotizing cells. The fact that fibrocytes are almost invariably found invading areas containing degenerating liver cells caused Hall and Ophüls¹⁹ to suggest that toxic substances which destroy the less resistant liver cells serve only to stimulate the more hardy connective tissue to active proliferation. References have been cited to show that simple destruction of liver tissue by a variety of methods does not, as a rule, lead to permanent fibrosis. Ordinarily the liver tissue regenerates, and temporary scarring later completely disappears.

Considerable variability was noted in the amount of cellular infiltration of the periportal connective tissue. In most instances, the greater proportion of cells consisted of small lymphocytes as in chronic cirrhosis.

In the cases of subacute cirrhosis, however, almost regularly a moderate number of polymorphonuclear leukocytes were present as well. In a few cases they were quite abundant (fig. 4), exceeding the number of lymphocytes.

Necrosis of the Liver Cells.—A prominent characteristic of the subacute type of cirrhosis is the presence of hepatic necrosis. In the chronic stage of Laënnec's cirrhosis ordinarily one may search in vain for necrotic liver cells. Perhaps a few scattered cells caught within the connective

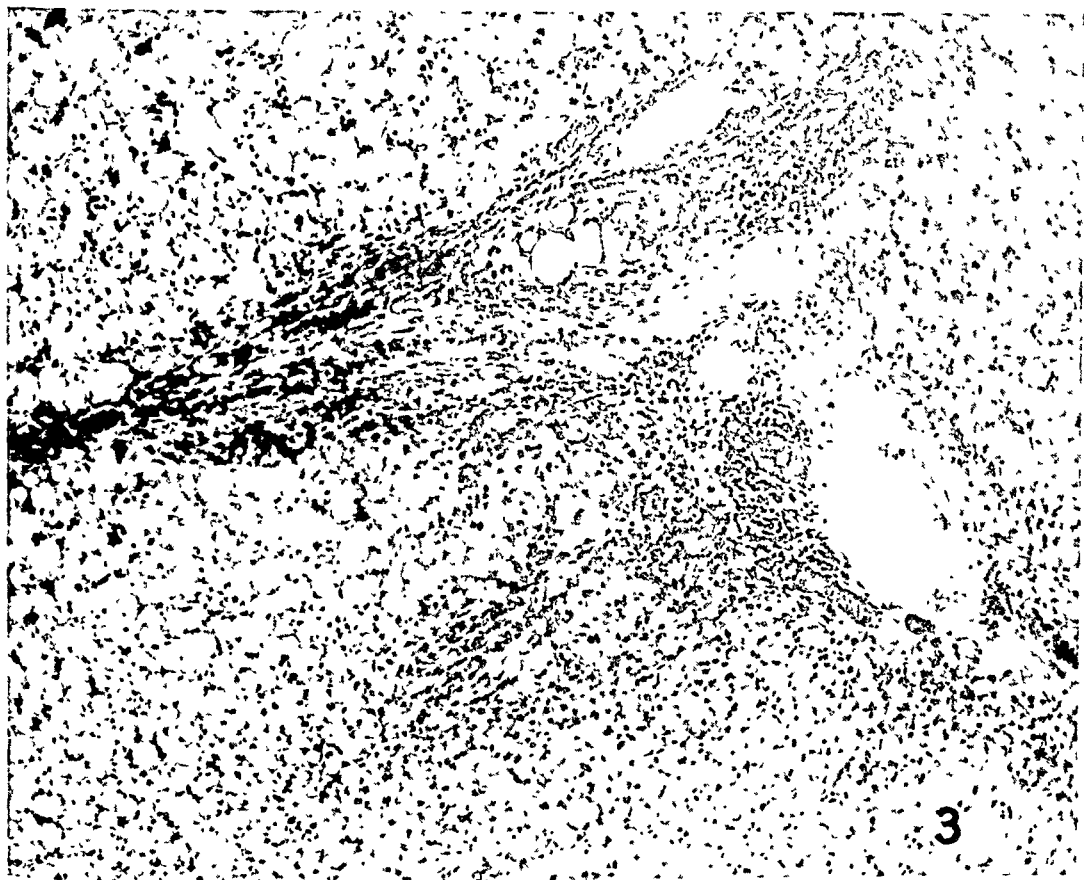


Fig. 3.—The patient was a Caucasian man aged 38 years, with chronic alcoholism. The liver weighed 2,080 Gm. The photomicrograph shows marked fatty infiltration of the liver, with cellular connective tissue growing out from the portal area into the surrounding parenchyma. Inflammatory cells infiltrate the connective tissue. Hematoxylin and eosin; $\times 120$.

tissue appear atrophic or actually necrotic. This is probably largely mechanical, however, and not the direct result of toxic action. In 24 of our cases, or 35.8 per cent, occasional necrotic or degenerating cells were seen along the borders of the periportal fibrous areas. In 21 cases, or 31.8 per cent, there were a moderate number of necrotic cells; in 18 cases, or 27 per cent, groups of necrotic liver cells were seen not only in the periphery but also in various positions within the lobule (fig. 5).

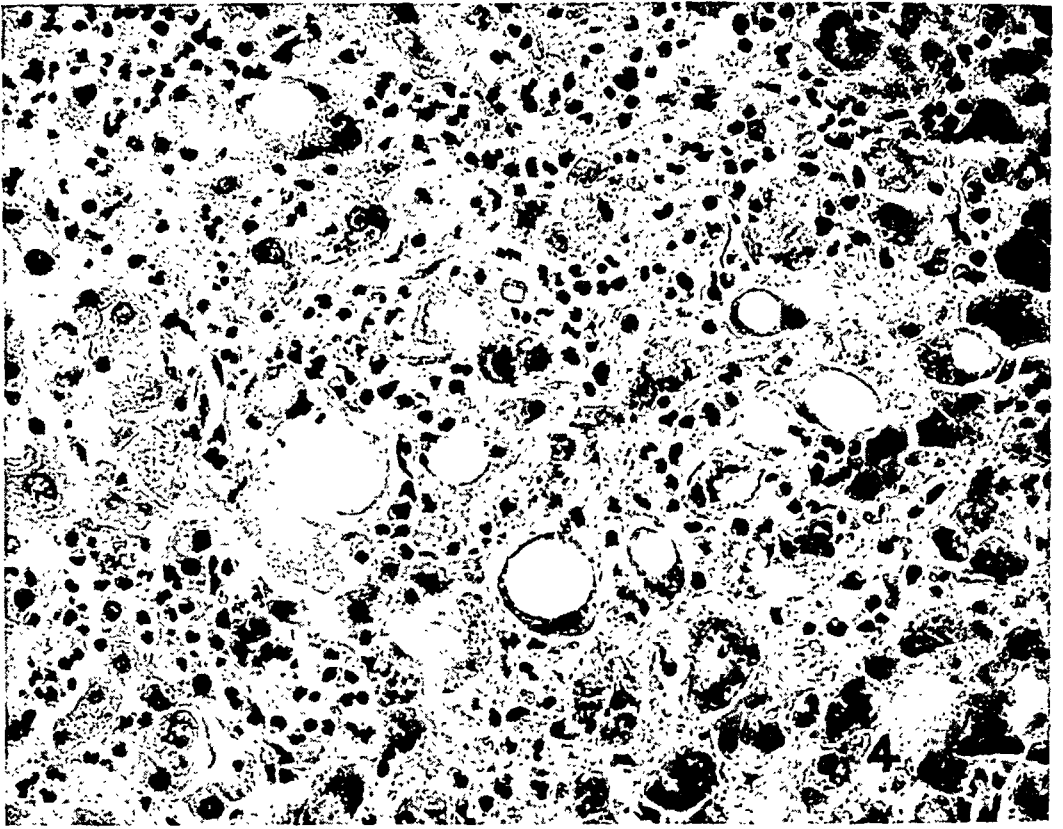


Fig. 4.—The patient was a Mexican man aged 39 years, markedly alcoholic. The liver weighed 3,300 Gm. The photomicrograph (high power) illustrates early proliferation of fibroblasts about necrotic liver cells. Many of the latter contain "hyalin." There is a diffuse infiltrate of inflammatory cells, many of which are polymorphonuclears. Hematoxylin and eosin; $\times 335$.

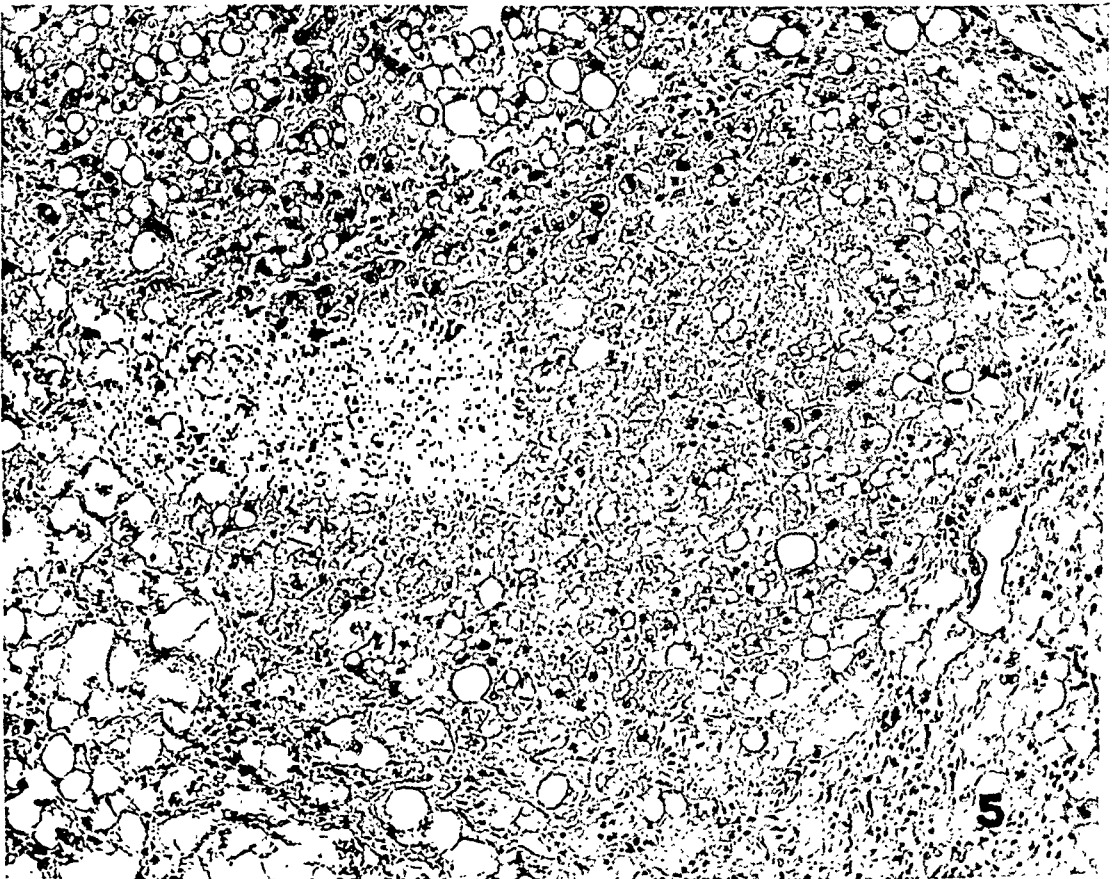


Fig. 5.—The patient was a Caucasian man aged 57, with chronic alcoholism. The liver was extremely fatty and weighed 5,000 Gm. The photomicrograph (low power) shows a large amount of fat, a large area of necrosis and a moderate increase of cellular fibrous tissue. Hematoxylin and eosin; $\times 100$.

In a few cases the liver showed a marked degree of necrosis. In general, the earliest changes in the degenerating liver cells appear in the form of either nuclear fading or pyknosis accompanied by vacuolation of the cytoplasm. The latter is due usually to hydropic degeneration. The cytoplasm swells, and numerous small round or polygonal clear spaces appear. Later, the nucleus disappears, and the cells, which may become greatly swollen, disintegrate (fig. 6). Other cells exhibit a peculiar lumpy hyaline appearance of the cytoplasm, which seems to represent a type

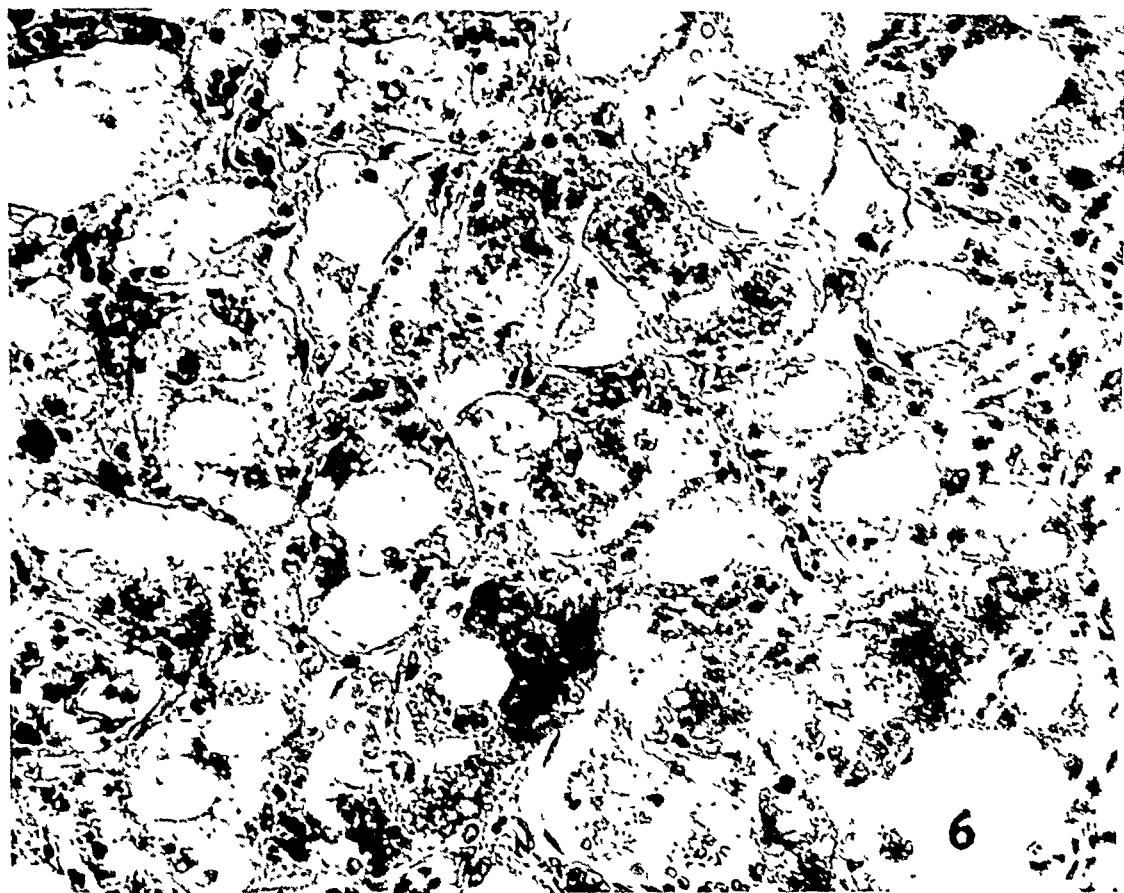


Fig. 6.—The patient was a Caucasian woman aged 39 years. She had chronic alcoholism and the Wassermann reaction was positive. The liver weighed 3,200 Gm. The photomicrograph (high power) shows a number of swollen, degenerating liver cells. Three large cells near the center contain masses of "hyalin" in their cytoplasm. Other cells show hydropic degeneration. Fibroblasts are beginning to appear about the degenerating cells. Polymorphonuclears are present. Methylene blue-eosin; $\times 335$.

of coagulative necrosis. The hyalin stains more deeply with eosin than the granular parts of the cytoplasm. Karyolysis and pyknosis of nuclei frequently accompany these changes, occurring at times within the same cells. Mallory²³ first described the hyaline changes as specific for alco-

23. Mallory, F. B.: Bull. Johns Hopkins Hosp. 22:69, 1911.

holic cirrhosis but does not so regard them at the present time. Fifty-five, or 80.88 per cent, of the livers in our series showed the "alcoholic" hyalin to some degree. In many instances it was abundantly evident (fig. 7). In 9 instances (13.2 per cent) the presence of hyalin was questionable. In some of these cases the stains were unsatisfactory, and material was not available for special stains. In 4 cases (6.2 per cent) hyalin was recorded as absent.

Fatty Infiltration.—Roughly, about three fourths of the livers in our series were fatty, and no doubt the increased fat content accounted for much of the increase in hepatic weight. Practically no fat was present

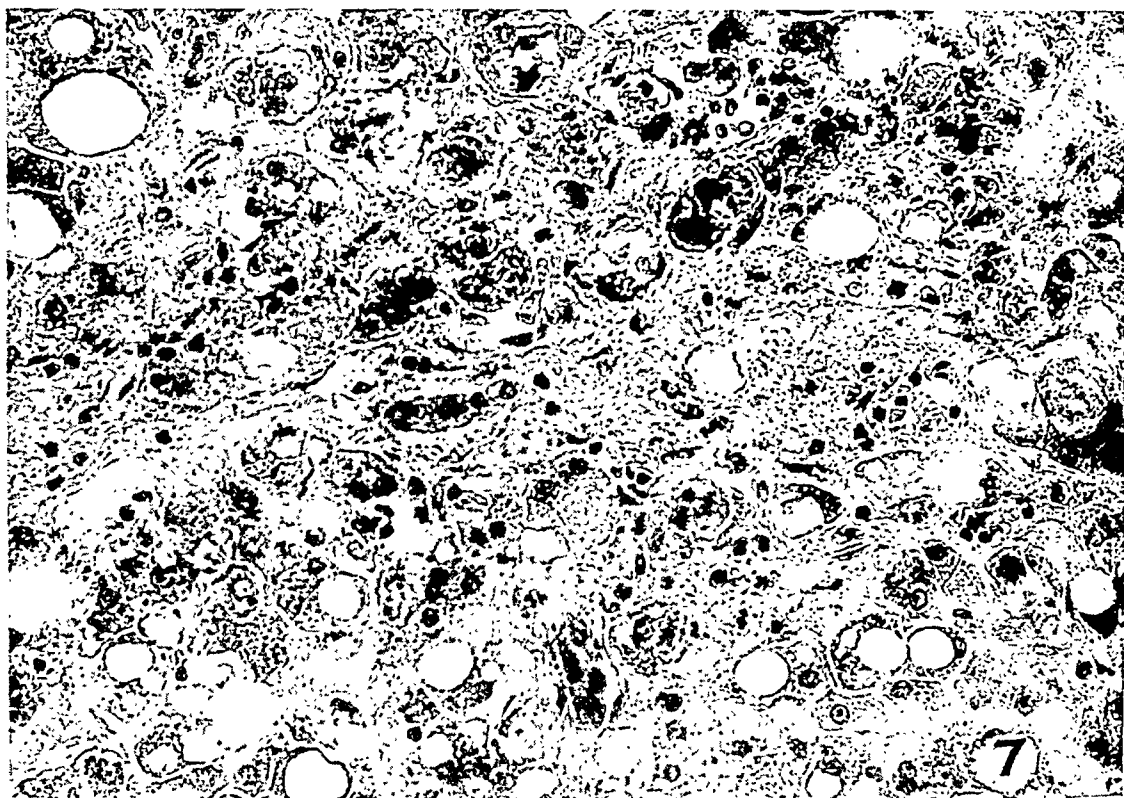


Fig. 7.—The patient was a Caucasian woman aged 56 years. She had chronic alcoholism. The liver weighed 4,400 Gm. The photomicrograph (high power) shows many necrotic liver cells, most of which contain lumpy masses of dark-staining "hyalin." There is more or less new connective tissue, and fibroblasts may be seen growing in among the dead liver cells. The paler areas are edematous. Some of the infiltrating cells are polymorphonuclears. Methylene blue-eosin; $\times 335$.

in the sections of liver in 5 cases, or 7.4 per cent; a small amount was seen in 17 cases, or 25 per cent, and a moderate amount in 15 cases, or 22 per cent; 17 livers (25 per cent) showed a marked fat content; while 14, or 20.6 per cent, were extremely fatty. Roughly, about 50 per cent of the livers contained a large amount of fat.

Damage to the liver cells by phosphorus, alcohol, bacterial poisons, starvation, diabetes and common wasting diseases, such as tuberculosis and malignant tumors, causes accumulation of fat in the liver. Observations at the autopsy table provide ample proof of this. Furthermore, the effects of alcohol and of starvation in producing fatty changes in the liver have been proved experimentally by a number of investigators—in the case of alcohol by Ruge,¹³ Friedenwald¹⁴ and Fahr¹⁵ and in the case of starvation by Rosenfeld,²⁴ Mottram²⁵ and Dible.²⁶

COMMENT

Analysis of the clinical data relative to possible etiologic agents in the 68 cases reviewed by us shows that chronic alcoholism was the factor common to the greater number of patients. We have found no report of a large group of cases showing so high an incidence of chronic alcoholism as in the present series (80 to 90 per cent). Evans and Gray stated that the incidence of cirrhosis in the Los Angeles County Hospital following repeal of the eighteenth amendment increased to three times that of the prohibition period. Their cases cover the prohibition years and the period following repeal up to May 1937. In spite of the decided rise after 1932, the total incidence of alcoholism was only 25 per cent. Boles and Clark² reported an incidence of 35 per cent. Mortality statistics for the registration area of the United States (quoted by Rowntree²⁷) show a drop of 50 per cent in the incidence of cirrhosis in this country during the prohibition era.

These and many other facts which cannot be included here relate alcoholism and cirrhosis so closely that an etiologic relationship must be seriously considered even though it is not yet proved.

There is a general feeling even among the proponents of the alcohol-cirrhosis relationship that some additional factor is necessary, or that special conditions must be complied with, before alcohol becomes effective in producing cirrhosis.

In our routine autopsies we have had the feeling from time to time that persons having chronic alcoholism who have at the same time a positive Wassermann reaction show more severe hepatitis than those whose hepatic disease is not complicated by syphilis. In the present series; of those livers in which the combination of alcoholism and syphilis was operative, 50 per cent show extreme fibrosis, with broad bands of fibrous tissue not confined to the periportal areas but in their extent and distribution suggestive of *hepar lobatum*. Of the remaining 58 cases, only

24. Rosenfeld, G.: *Ergebn. d. Physiol.* **1**:651, 1902.

25. Mottram, V. H.: *J. Physiol.* **38**:281, 1909.

26. Dible, J. H.: *J. Path. & Bact.* **35**:451, 1932; **38**:269, 1934.

27. Rowntree, in discussion on Boles and Clark.²

20 per cent (in all of which the Wassermann reaction was negative or not recorded) exhibit such extensive fibrosis.

Jaundice was present in 50 per cent of our patients. Omitting the 7 who had terminal bronchopneumonia, we find that 10 patients had acute infections. Twenty-four patients showing clinical jaundice are recorded as having moderate to severe necrosis of the liver cells. In the latter group the condition of the liver may be considered quite definitely the result of the toxic action of alcohol. In 6 instances necrosis was called marked to severe (+ + + to + + + +) on histologic grounds, but clinical jaundice was not recorded. Ascites was found in the same percentage (60.3) in our group as reported by Evans and Gray¹⁸ for the cases of chronic cirrhosis. Fatal hemorrhage from ruptured esophageal varices occurred in 13.9 per cent of their cases and in 20 per cent of ours.

Enlargement of the liver was the most striking anatomic finding in our series. The average weight was 2,760 Gm., which is approximately double the weight of the average hobnail liver. Fibrous tissue is usually abundant in the liver in the cases of subacute cirrhosis but is less mature than in the cases of chronic cirrhosis. Fibroblasts are evident, especially about necrotic liver cells.

A large infiltrate of fat was found in about 50 per cent of the livers in our series. Another 20 per cent were considered moderately fatty.

Connor²⁰ recently reviewed the subject of fatty infiltration of the liver in relation to alcoholism and cirrhosis. He cited adequate authority for the statement that poisons such as ether, chloroform, carbon tetrachloride and alcohol interfere with carbohydrate metabolism and so with the proper oxidation of fats. His excellent report should be consulted for a more detailed discussion of fatty infiltration of the liver.

That dietary deficiency plays a prominent role in chronic alcoholism and in alcoholic cirrhosis has been emphasized recently by Romano.²⁸ Of 131 patients with alcoholism studied, 77, or 58 per cent, presented some degree of neuritis, and 61, or 79 per cent, of those showing neuritis had a history of inadequate food intake previous to admission. Sixty-one per cent showed partial improvement and 32 per cent showed complete recovery on specific vitamin therapy.

Patek²⁹ also studied the matter of dietary deficiency in patients with alcoholism. In 9 of 13 patients the caloric intake was very low. On a diet high in calories plus specific vitamin therapy 10 patients who survived showed decided improvement.

Dr. W. L. Adams, one of the medical house officers of the Los Angeles County Hospital, assures us that a diet high in calories plus high vitamin therapy causes rapid decrease in the size of the liver and relief of

28. Romano, J.: *Am. J. M. Sc.* **194**:645, 1937.

29. Patek, A. J., Jr.: *Proc. Soc. Exper. Biol. & Med.* **37**:329, 1937.

symptoms in patients suffering from subacute cirrhosis. The diagnosis in these cases has been definitely confirmed by peritoneoscopic examination and biopsy of the liver. All of the patients have chronic alcoholism. A person who drinks a pint or more of whisky per day must find it difficult to assimilate an adequate amount of food. Since, according to Sollmann,³⁰ 1 cc. of whisky produces 4 calories of energy, a pint of whisky (50 per cent) would provide approximately 2,000 calories of energy daily. Besides the increase in actual calories, there is likely to be loss of appetite due to gastritis, disturbance of liver function, vitamin B deficiency and other abnormal conditions. Furthermore, the periodic drunkard, who goes on a prolonged debauch, must be for days at a time inadequately nourished or actually in a fasting state. It is common knowledge that a liver so thoroughly depleted of glycogen is vastly more susceptible to damage than one adequately protected by a store of glycogen. It is under these conditions that repeated large doses of alcohol are likely to prove damaging. Not only does fat tend to increase, markedly in such livers as a result of the interference with carbohydrate metabolism and with oxidation of fats, but marked anoxemia develops in the liver and the body as a whole, according to Connor.²⁰

Corroborative of the foregoing observations, the recent report of Von Glahn and his co-workers¹² is interesting. They found that a diet high in carbohydrate afforded their rabbits marked protection against developing cirrhosis. A group of 26 rabbits received daily amounts of arsenates ranging from 1.86 to 2.4 mg. on a diet of white bread and uncooked peeled white potatoes. Only 2 animals showed cirrhosis, which was of a mild degree, while 24, or 91 per cent, had no increase of hepatic connective tissue. By feeding carbohydrate-poor diets, these workers, using copper, lead and sodium arsenates, had obtained cirrhosis in more than 90 per cent of their animals. It appears that a diet rich in carbohydrates protects the liver from the injurious effect of arsenates when these are given in small doses.

Bollman and Mann³¹ cited experiments showing variations in the susceptibility of fatty livers of dogs to alcohol when the glycogen content of the livers was greatly reduced. With feeding of a fat diet, the fat content of the liver by the fourth week had risen to 40 per cent. The glycogen content decreased at the same time to 0.2 to 0.3 per cent. Alcohol of 95 per cent strength fed in doses equivalent to 1.5 cc. per kilogram of body weight to dogs in the aforementioned condition caused coma and intoxication lasting one to two hours. In animals with 50 per cent fat and 0.1 per cent glycogen in their livers a slightly increased dosage (2 cc. per kilogram) usually proved fatal. A dosage of 1.5 cc.

30. Sollmann, T.: *Manual of Pharmacology*, Philadelphia, W. B. Saunders Company, 1932, p. 706.

31. Bollman, J. L., and Mann, F. C.: *Am. J. Physiol.* **116**:214, 1936.

of 95 per cent alcohol per kilogram in dogs with normal livers caused them to become only momentarily intoxicated. A change from a fat to a carbohydrate diet restored the excessively fatty livers to normal within a week.

LeCount and Singer¹⁶ emphasized the serious effects of a reduction in liver glycogen in chronic alcoholism. They reported a number of cases of sudden death in alcoholic persons who had extensive replacement of the liver by fat. The postmortem examinations revealed little else than large fatty livers.

Not all of the livers in our series were fatty—30 per cent contained relatively small amounts of infiltrating fat. Crandall and Ivy,³² in a recent review on applied physiology of the liver, stated that in hepatic disease the liver requires increased amounts of sugar in the form of dextrose. They give two reasons for this: In the first place, there is a decreased capacity for storage of glycogen; in the second, the diseased liver is incapable of producing sugar from noncarbohydrate sources (lactic acid, amino acids and glycerol) in order to meet the normal requirements of the body. It appears, therefore, that in alcoholic livers in which excessive fat does not accumulate, there is, nevertheless, increased need of abundant sugar because of the decreased capacity for storage of glycogen and the incapacity of these livers to produce glycogen vicariously. Histologically, all of the livers are diseased, even when there is little or no excess of fat. Connor²⁰ believes the fatty condition of the liver to be the earliest stage in the development of cirrhosis and that later in many cases excess fat disappears as the fat depots are depleted.

Bloomfield²² stated that after the onset of symptoms cirrhosis often develops rapidly. There is a long period, often one of years, in which the disease is latent and no symptoms relative to the liver are observed. After the advent of symptoms, the patient may survive for only a few months. Eight of his patients lived less than ten months after symptoms developed, and 2 of these lived only one month. The majority lived ten to forty months. Bloomfield's observations explain, in part at least, the paradox of the rapid increase in mortality due to cirrhosis, a disease usually considered exceedingly chronic, following the repeal of the eighteenth amendment. Resumption of heavy drinking may have precipitated many persons with alcoholism into rapidly fatal cirrhosis.

The question may properly arise as to why some patients die during the subacute phase of alcoholic cirrhosis. In this connection the following observations appear to be important:

(a) About 25 per cent of the patients died of acute complicating infections, due largely, no doubt, to marked lowering of their resistance by chronic alcoholism.

32. Crandall, L. A., and Ivy, A. C.: *Surgery* 3:815, 1938.

(b) Twenty per cent showed direct effects of alcoholism plus dietary deficiency—half of these had pellagra or peripheral neuritis; in the remaining half either an alcoholic psychosis or delirium tremens developed. Two additional patients had both delirium tremens and pellagra.

(c) Hepatic insufficiency is probably an important factor, since 50 per cent of the patients were jaundiced, and 14 per cent showed hepatic necrosis as a cause of death at the postmortem examination. All of the latter were jaundiced.

(d) Twenty per cent in our series died of gastric hemorrhage as against 13.9 per cent in the cases studied by Evans and Gray. Therefore portal hypertension appears to be an important cause of death in subacute as in chronic cirrhosis.

SUMMARY AND CONCLUSIONS

A study was made of 68 cases of subacute or progressive alcoholic cirrhosis. The cases were selected from among 12,000 in which autopsies were performed at the Los Angeles County Hospital during the period from 1931 to 1938. They were selected on the basis of liver weights (2,000 Gm. or over) and the presence of hepatic necrosis, portal cirrhosis showing active proliferations of connective tissue, and so-called "alcoholic" hyalin.

Polymorphonuclear leukocytes as well as lymphocytes infiltrated the fibrous tissue. The average weight of the 68 livers was 2,760 Gm. Fifteen weighed between 3,000 and 4,000 Gm.; 6 weighed between 4,000 and 5,000 Gm.

At least 80 per cent of the patients had chronic alcoholism. Only 1 of the 68 patients denied having used alcohol. Jaundice was present in 50 per cent of our patients. In 24 instances the icteric index was 50 units or higher. Approximately 50 per cent of the livers contained excessive amounts of fat. Fatty infiltration was considered moderate in 20 per cent and slight in 30 per cent. Syphilis complicating alcoholic cirrhosis appears to produce a more profound fibrosis than is usually seen in the latter condition alone.

Alcoholism was the outstanding, most common clinical factor in this group of cases. Since the group was selected on anatomic grounds, it appears that the relationship is of real importance. The patient with chronic alcoholism suffers from low intake of carbohydrate, greatly diminished storage of glycogen, fatty replacement of the liver and deficient intake of vitamins. These conditions render the liver especially vulnerable, so that continued abuse of alcohol is followed by necrosis of liver cells and fibrosis, which in the susceptible patient after years results in cirrhosis.

We believe that in the majority of cases the enlargement and fatty infiltration of the liver constitute the first development, that this passes

imperceptibly into the subacute phase of cirrhosis, to be followed later, if the patient survives long enough, by the appearance of the small hobnail liver. A small percentage of the patients die during the subacute stage as a result of complicating infections, dietary deficiency diseases, hepatic insufficiency, portal hypertension, alcoholic psychoses and other disorders.

We believe the term "alcoholic cirrhosis" has a firm basis in etiology. Much of the confusion regarding this subject has been due to the study of cases of chronic Laënnec type among which the cases of cirrhosis due to alcohol cannot be distinguished readily from cases due to other causes.

THE NORMAL, THE ACROMEGALIC AND THE HYPERPLASTIC NEPHRITIC HUMAN NEPHRON

A FURTHER CONSIDERATION OF THE PLASTIC RECONSTRUCTIONS
OF LOUIS A. TURLEY

ALLAN L. GRAFFLIN, M.D.

BOSTON

Some years ago I was struck by the great beauty of three wax models of the tubules of the human kidney which I found preserved in the collection of the Department of Anatomy, Harvard University Medical School. These models, now over twenty years old, were made by Louis A. Turley, then a graduate student at Harvard University, now professor of pathology in the School of Medicine of the University of Oklahoma. They represent (1) the normal nephron, (2) the glomerulus and proximal convoluted tubule in acromegaly and (3) the hyperplastic nephron in chronic nephritis. Models 1 and 3 formed the basis of Turley's doctoral thesis¹; in the thesis photographs of the two models and several other illustrations are given. This thesis was not published in its original form. In a subsequent paper² Turley discussed his findings for the normal and for the nephritic nephron, without, however, mentioning or giving any reproduction of the models in question. The model of the proximal tubule in acromegaly has never been described, either in the thesis or elsewhere.

These three models,³ all of them beautifully and painstakingly executed, offer data of great interest and value to investigators of the finer structure of the kidney. The model of the normal nephron represents the only instance, up to the present time, in which the reconstruction of an essentially complete adult mammalian nephron—including the loop of Henle—has ever been successfully accomplished. The model

From the Department of Anatomy, Harvard Medical School.

1. Turley, L. A.: Studies by Reconstruction of the Compensatory Changes in the Tubules in Chronic Diseases of the Kidney, Thesis, Division of Medical Sciences, Harvard University, 1916. (This thesis is on file in the library of Harvard University.)

2. Turley, L. A.: *Ann. Med.* 1:401, 1920.

3. The models are numbered as follows in the model collection of the department of anatomy of Harvard University: normal nephron, no. 201; hyperplastic nephron in chronic nephritis, no. 202; glomerulus and proximal convoluted tubule in acromegaly, no. 203.

of the proximal tubule in acromegaly is likewise unique. The model of the hyperplastic nephron in chronic nephritis antedates by many years the recent model of Oliver and Lund ⁴ and confirms, and to some extent supplements, their findings and the findings in the maceration studies of Oliver and Luey ⁵ and Loomis.⁶

Under the circumstances, it has appeared to me eminently desirable that a fairly complete series of illustrations of Turley's models, accompanied by a description of his findings, should be made available to other interested workers. I requested Dr. Turley's permission to carry out such a project, and this permission he freely gave. Furthermore, he has cooperated with me to the fullest extent in supplying necessary additional data which were not given in his thesis or in his subsequent publication. I wish to emphasize that my activity has been confined to the study of the models themselves and to the supervision of the illustrations. Mr. Leo Talbert made the photographs, and Miss Etta Piotti made the line drawings. Dr. Turley's findings—based on the models and supplementary studies—will be given in his own words, taken largely from his thesis.

Turley's reasons for undertaking his study are best explained in the introductory paragraphs of his thesis ¹ (pages 1 and 2):

In the studies that have hitherto been made of the changes in the tubules in chronic diseases of the kidney, investigators have turned their attention to the atrophic and degenerative changes only. In no account of the changes in the kidney tubules has any mention been made of any but those of a retrogressive nature, except that Dr. Councilman in his lectures to his students, and in the protocols of autopsies, speaks of hyperplasia of the tubules in some cases. These studies were undertaken to ascertain whether or not the apparent hyperplasia of some of the tubules was really an hyperplasia and if so, to what extent did hyperplasia, or compensatory change take place. And to this end it was decided that the method of studying serial sections, tracing one tubule throughout its length, and making a wax reconstruction of the tubule was the one that was best suited to show the exact amount of change that had taken place.

But before any exact knowledge could be gained of the changes in the pathological tubule, it was necessary to make a careful study of the normal tubule in the adult kidney. Huber ⁷ in this country, and Stoerk ⁸ in Germany have carefully worked out the development of the kidney tubule up to the time of birth and have made excellent models of the tubules in various stages of their development, showing the relative size, shape, and the arrangement of the parts. But just what additional changes may have taken place after birth in the complexity of the convoluted portion of the tubule or in the loop of Henle, the diameter of the

4. Oliver, J., and Lund, E. M.: Arch. Path. **15**:755, 1933.

5. Oliver, J., and Luey, A. S.: Arch. Path. **18**:777, 1934.

6. Loomis, D.: Arch. Path. **22**:435, 1936.

7. Huber, G. C.: Am. J. Anat. (supp.) **4**:1, 1905.

8. Stoerk, O.: Anat. Hefte **23**:283, 1904.

normal tubules, whether or not the diameter was constant for one tubule throughout any one of the anatomical divisions, the height of the epithelium and other points that go to make up an exact knowledge of one of these structures has not, so far as the author has been able to find out, ever been made. Therefore, it was necessary to do considerable work on the normal tubule in order to have a basis for comparison in a consideration of the conditions found in the pathological material studied.

Turley was unfortunately not acquainted at that time with the extensive studies of the normal human kidney which had been published some years previously by Peter.⁹ In the course of this work Peter reconstructed his now classic, though incomplete, model of the normal adult human nephron. Turley's and Peter's models and observations admirably supplement each other, and will be discussed at greater length later.

METHOD OF RECONSTRUCTION

All three models were reconstructed from serial sections of paraffin-embedded material, stained with hematoxylin and eosin. The following description of the method employed is taken from Turley's thesis¹ (pages 2 and 3) :

Drawings of the tubule in the various sections were made with the aid of the camera lucida. At first some difficulty was met in getting the exact relation of the sections of the tubule as it appeared in the various sections, and a number of means were tried to obtain this exact relation. The common method of students of embryology, that of using the outline of the dorsum of the embryo as a guide, was impossible for the reason that the capsule of the kidney is not itself constant on either surface, in the mounted specimen. Ritzer lines were tried and these were found to be, if anything, less reliable than the capsule of the kidney for the reason that in the hands of the author it was impossible to get the sections mounted without distorting the Ritzer lines. Finally the plan to use other structures in the kidney as guides was hit upon and by taking several at a time it was found that the position of any tubule could be maintained through as many sections as it might pass. For this purpose the structures used were glomeruli, blood vessels, and the collecting tubules. And by taking two glomeruli at a time, a collecting tubule and one of the larger vessels it was not difficult to find and maintain the relation of the various sections of any tubule in passing from section to section.

The magnification that was found best suited to this work was two hundred diameters. If a larger magnification were used it would make the model too large and cumbersome, and if a lower magnification were used it would both add to the difficulty of following the tubule and would make the model so delicate that the reconstruction of the entire system into one model would be next to impossible. Therefore all drawings were made with a magnification of two hundred diameters and the wax plates for the reconstructions were made two hundred times the thickness of the sections of the kidney from which the drawings were made.

9. Peter, K.: Die Nierenkanälchen des Menschen und einiger Säugetiere, in Untersuchungen über Bau und Entwicklung der Niere, Jena, Gustav Fischer, 1909, no. 1, p. 1.

The wax plates were made of bees' wax by the method of pouring an accurately weighed amount of melted wax on hot water in a vat of exact dimensions. After cooling, these plates were measured with a micrometer to be sure that they were of the required thickness and no plate was used that was under or over the necessary thickness.

THE NORMAL NEPHRON

Material.—For this purpose I was fortunate enough to find a kidney, which, although it was a little under the normal weight, was nevertheless the site of so few pathological changes that the "brush like inner border" of the epithelium of the tubules was still preserved.¹⁰ Material from this kidney was cut into a series of three hundred sections seven microns thick and mounted in order . . .

This series of sections was studied carefully to find as near as possible an average tubular system. One was finally selected about midway between the capsule and the arcuate vessels, and one from which the tubule left the glomerulus on the side toward the pelvis of the kidney which may be said to be the traditional point of departure of the tubule from the glomerulus, although it was found that the tubule may leave the glomerulus at any point even directly toward the capsule of the kidney irrespective of the position of the system in the cortex. [Thesis,¹ page 2.]

Observations.—By methods outlined above a normal tubule was studied and reconstructed and the results compared with serial drawings of other tubules to ascertain how nearly the reconstructed tubule represented an average tubule, and these facts were used as a basis of conclusions in regard to conditions found in the pathological material studied. A description of a normal kidney tubule, as found by the author, is as follows:

The tubule, from whatever angle it leaves the glomerulus, soon passes toward the capsule, following a tortuous course and continues to bend and turn on itself until it is some distance from the glomerulus. It then turns toward the glomerulus in a more direct course and passes the glomerulus in the descending limb of the loop of Henle. In its course toward the capsule each bend or turn that the tubule makes does not carry it further from the glomerulus, but there may be said to be major and minor turns. That is, the turns lead away from and then toward the glomerulus, and then away again, so that, for example, turn eleven and turn twenty-five are both next to the capsule of Bowman and the turns between eleven and twenty-five are farther away from the glomerulus than either of the two mentioned. It would seem that at some stage in the development of the tubule there were no turns between what is now turns eleven and twenty-five but that the tubule had arched above the glomerulus as a simple bend, and that later the system had become more complex and that the newer turns which had arisen as a result of the lengthening of the tubule had carried part of the tubule farther away from the glomerulus than a part that was really more distal. But eventually the tubule reaches a considerable distance from the glomerulus and then turns toward it again in a more direct direction. In this way the average number of turns in the proximal convoluted tubule, to the point where it again reached the level of the glomerulus on its way to the loop of Henle is forty.

The loop of Henle descends in as nearly a direct direction as the other structures in the vicinity will permit, with the exception that it will sometimes, in some

10. The kidney material for the reconstruction was taken in the course of autopsy no. 15-46 at the Peter Bent Brigham Hospital.

tubules, turn almost at right angles across a cortical ray and turn as sharply again toward the medulla, until it reaches the arcuate vessels. From these structures to the return in the loop it follows a more zigzag course. The thin part of the tubule is comparatively short. The turn is abrupt and the ascending arm of the loop follows a similar, and in general a parallel course to the descending limb of the loop. But it was found that it is not at all uncommon, especially in those tubules that pass below the arcuate vessels, for the ascending limb to have an "S" shaped bend in the medulla before passing the arcuate vessels on their way back to the glomerulus. The ascending limb follows, in general, a more direct course than the descending limb, and in every case passes the glomerulus near the afferent artery. So regular is this that anyone can be sure that he has the ascending limb of the loop of the tubule to any glomerulus by finding the afferent artery to the glomerulus and taking the ascending tubule found there without following out the whole system.

The distal tubule makes few turns, eleven in the system modeled, until it arches to join the collecting tubule.

The shape and diameter of any tubule is not constant for all parts of the system, or even the proximal convoluted part. The shape of the tubule will vary with the pressure of the other turns of itself, or with other structures. The diameter of the tubule in this part is in general greater near the glomerulus, and remains fairly constant for a considerable distance, then there is some reduction in the diameter which, although it is gradual, takes place in a comparatively short space. This reduced diameter is then constant and continues into the descending arm.

In the descending arm there is a gradual reduction in the diameter of the tubule until it reaches a somewhat smaller size which it maintains until it goes over into the thin part of the arm.

The diameter of the ascending arm is constant, or nearly so, throughout its length. That is, there is no region in which the diameter is larger or smaller in general, although it may vary from place to place.

The diameter and shape of the distal convoluted tubule is quite variable, but in general it is greater as it approaches the junctional part, when it again is reduced in diameter to a slight degree.

The measurements of the various parts are as follows: The diameter of the proximal convoluted tubule averages 60 microns to the basement membrane on either side and it varies between 75 and 45 microns; the upper part of the descending arm is 50 microns and the reduced diameter is 45 microns in the lower part; the average diameter of the ascending arm is 37.5 microns; and the distal convoluted varies between 40 and 50 microns.

As the tubule leaves the glomerulus the epithelium changes suddenly from a simple squamous to a high cuboidal, or glandular cuboidal form. The height of this epithelium is 17.5 microns on an average and the free ends of the cells are all on a level so that the lumen of the tubule is sharply defined. The height of the epithelium remains constant throughout the proximal convoluted tubule. The height of the epithelium in the descending arm is 15 microns in the upper part and is reduced very little until the thin part of the arm is reached. At the distal end of the thin part, the epithelium takes on the character of the ascending arm of the loop, so that structurally and functionally the distal part of the system may be said to begin at the distal end of the thin part of the loop of Henle.

From the figures given above it can easily be calculated what the square area of the epithelium in any part of the tubule is. Also what is the perimeter of the lumen of the tubule in any part. Thus the area of the epithelium in an average cross section of the proximal convoluted tubule is 2336.565 square microns, and the perimeter of the lumen at the same point is 78.54 microns. [Thesis,¹ pages 4 to 7.]

THE GLOMERULUS AND PROXIMAL CONVOLUTED TUBULE IN ACROMEGALY

As stated, the model of the glomerulus and proximal convoluted tubule in acromegaly has never been described by Turley, either in his thesis or elsewhere. The model was made over twenty years ago, and Dr. Turley no longer has any record of the autopsy number or any complete description of the kidneys as observed at autopsy. In answer to my request he has, however, supplied the following information:

Dr. Councilman asked me to make a model of the glomerulus and proximal portion of the nephron in an attempt to explain a condition which could not be determined by gross or microscopic examination of the kidney. Dr. Councilman's statement was as follows: "Each of these kidneys weighs twice as much as a normal kidney should, but there is no evidence either grossly or microscopically which will explain the increased size." I remember that at the time microscopic examinations of sections of these kidneys did not show any abnormal histologic character, so that the description would be that of a normal glomerulus and tubule. . . . The material came from Dr. Cushing's clinic for the study of pituitary disturbances. . . . My studies showed that the proximal tubule was twice the length, while the diameter was the same as that of the normal. A comparison of this model with a model of the normal tubule will show that the general structure of the acromegalic kidney was normal, but that the tubule was twice the length of the normal, which accounts for the increased size of the mass in the model.

THE HYPERPLASTIC NEPHRON IN CHRONIC NEPHRITIS

Material.—In selecting material for this study, the material at the Peter Bent Brigham Hospital was gone over carefully and all of the material from chronic diseases of the kidney was studied, and from this several cases were selected, the basis of selection being that there was enough in any one piece of tissue to make a series of at least a hundred sections that would show as much as possible of a section from the capsule to the pelvis. From the cases selected one of them was chosen as a type because the tubules functioning at death had not undergone any great degenerative changes so that the character of the epithelium was as near normal as possible.¹¹

The combined weight of the kidneys is 195 grams. They are similar, the most conspicuous thing about them being the small size. The capsule strips with some difficulty, tearing off with it small portions of the underlying tissue and revealing a rough granular surface made up of numerous small elevations from .5 mm. to 1.5 mm. in diameter. In some places this rough appearance is less marked and the tissue seems to be replaced by cicatrization. The kidneys are of a

11. The kidney material for the reconstruction was taken in the course of autopsy no. 14-1 at the Peter Bent Brigham Hospital.

pale color, with red lines in the depressions. On section the consistence is found to be somewhat increased, the surface is extremely pale, of a yellowish to purplish grey color, with a few fine red lines and spots in the cortex and the same in the pyramids. The whole surface is of a rather uniform color, the markings are very indistinct, the line between cortex and pyramids being hard to make out in certain places, while in others it is fairly definite. The average thickness of the cortex is 4 mm., average length of pyramids 15 mm. Ureters and seminal vesicle normal.

Section stained for fat shows small amount of fat, sometimes represented in the desquamated epithelium lying in tubules or in the thin attached degenerated epithelium. The hematoxylin and eosin sections show a generalized glomerular nephropathy, every degree of the lesion of the glomeruli represented up to complete destruction. The tissue is well preserved and the character of the lesions is evident. One of the most striking things in the section is the seeming absence of the glomeruli, due to the enormous degree of complete destruction. In certain of them there is a great increase in the covering epithelium with destruction of the vessels. A formation of hyaline connective tissue involves large areas of the organ. The tubules show a number of conditions; in the connective tissue masses, they are represented as very small epithelial masses or have disappeared; in other places the tubules are dilated and the epithelium so atrophic as to resemble pavement epithelium; many tubules show areas of extensive desquamation of such thin cells, and in others there are piled up masses of degenerated epithelium. This cannot be regarded as an artefact on account of the freshness of the autopsy and the care in taking the specimen. There are certain of the glomeruli in which the damage is much slighter and there are areas of tubules with the epithelium but little changed and which undoubtedly represent areas of hyperplasia. So it will be evident that these kidneys represent a chronic diseased condition where there is a compensatory change.

A series of one hundred and eighty-nine sections, 8 microns thick, was made from a piece of one of these kidneys, and it was studied and modeled in the manner and by the same means as described above for the normal kidney, and the following changes were found to have taken place. [Thesis,¹ pages 7 to 9.]

Observations.—The tubule remains the normal diameter for some distance from the glomerulus, then there is a gradual though quite sudden enlargement until the diameter is doubled or even larger in some places. The average diameter of these hyperplastic tubules was found to be 115 microns on an average, and some were found that were 140 microns in diameter. The tubules were greatly lengthened, one having 95, and one 106 turns in the proximal convoluted part to the point where the tubule passed the glomerulus on its way to the loop of Henle. In some of the single sections the diameter of the tubule was even greater, but by following it through the series so that the diameter vertical to the plane of the section was found it proved to be much less, hence the perimeter of the tubule at that point is not greater than at some point where the enlargement is apparently not so great.

It might be suggested that the apparent increase in the length of the tubule was due to the union of two or more tubules after the glomerulus had ceased to function. And it has been suggested by Felix¹² that in the development of the

12. Felix, W.: The Development of the Urinogenital Organs, in Keibel, F., and Mall, F. P.: Manual of Human Embryology, Philadelphia, J. B. Lippincott Company, 1912, vol. 2, p. 752.

kidney such unions do take place, though sufficient evidence of this suggested phenomenon has not been produced. And against such an assumption in the pathological case is the fact that in the group of hyperplastic tubules there is no evidence that any other glomeruli than the ones now present have ever existed. Students of pathology will remember that no matter how atrophied or fibrosed a glomerulus may become its location can always be found. So the conclusion must be drawn that the apparent increase in the length of these tubules is a real increase and represents an hyperplasia of a compensatory nature.

The epithelium of the tubule as it leaves the glomerulus is reduced to a squamous form, and this change extends for a considerable distance along the tubule, to the twelfth turn in some cases studied. Otherwise the epithelium is apparently normal in character. The average height of the epithelium is 17.5 microns, and the free border of the cells is, in general, on a level although apparently it is somewhat ragged. This apparent ragged border is due in most cases to the adherence of a granular deposit in the lumen of the tubule.

By the same calculations as were made in the case of the normal tubule, the area of epithelium in any cross section of the tubule is 5085.465 square microns or more than twice that of the normal tubule, and the perimeter of the lumen of the tubule almost four times as great. Add to these facts the greatly increased length of the hyperplastic tubule and we see that there is really an enormous increase in the functioning capacity of one of these tubules over that of the normal tubule.

The most remarkable change was found in the descending arm of the loop of Henle. Instead of running the usual straight course, it was turned on itself in sharp angles in both planes at right angles to its long axis, making in all 79 turns. It was greatly enlarged, the average diameter was nearly as great as that of the hyperplastic convoluted portions, being from 75 to 95 microns, and the epithelium was of the same character in all respects as that of the convoluted portion. And the perimeter of the lumen was as great as the outside perimeter of the epithelium of the normal tubule.

The figures given above are only averages, as the diameter of the tubule at various points was by no means constant either in the convoluted portion or in the loop. There were increases in size at some points that amounted almost to cysts and at other points the tubule was not much above normal. The increase in size at the various points seemed to depend on the pressure of the surrounding tissues. But taking it as a whole the above figures are a fair average of the enlarged condition.

Unfortunately it was not possible, in any of the series of sections that were obtained, to follow the tubule around the turn in the loop or even to find the thin part of the arm for the reason that the material was taken at random at the time of autopsy without any reference to making any use of it but for the common laboratory uses of diagnosis. In one case the specimen did not include all of the tissues from the capsule to the pyramid, and in another the specimen was too thin so that the tubules, running at an angle to the plane of the knife in taking the specimen, did not run the entire depth of the specimen. Therefore, this account does not include an entire tubule, and it must be left until the proper material can be secured before making it complete.

The changes in the ascending arm of the loop were slight in comparison to those that were found in the descending arm. The diameter was somewhat increased, being on an average 50 microns, and it was as constant as the diameter

of the normal ascending arm. The arm was also somewhat bent and turned to follow, in general, the course of the descending arm. The epithelium was of the same character as that of the normal distal convoluted tubule, taking a more dense and uniform stain than that of the normal epithelium of the ascending arm.

The distal convoluted tubule was enlarged, being 70 microns on an average. The number of turns was not increased or but by few more turns than in the normal. The epithelium was the same in character and thickness as the normal but it was in some places in some of the tubules, thrown into folds or rugae.

After completing the study of the tubules in this case, the tubules in other cases were studied by the examination of serial sections and making serial drawings. The same changes as described above could be traced in all of the cases of chronic disease of the kidney that were available. However, the changes were not so great in all cases, and the retrogressive changes in the hyperplastic tubules in some cases were great enough to almost mask the compensatory changes.

By comparison of the normal with the functioning tubules in chronic disease of the kidney the conclusion may be drawn that there is a compensatory change. This change is greatest in the descending arm of the loop of Henle for the reason that it departs the most widely from the normal. The change is that of an increase in the length of the tubule system and an increase in the epithelium at all points, which results in an increase in the diameter of the tubule. [Thesis,¹ pages 9 to 12.]

The thesis contains no histologic description of the glomerulus of the particular hyperplastic nephron which was reconstructed, but Dr. Turley has supplied such a description for inclusion in this report, as follows:

There is a slight increase in the subcapsular space, which contains a small amount of precipitated albuminous material and a few desquamated cells. The lobulation of the glomerular tuft is still apparent. In most of the lobes the intercapillary spaces have been obliterated; in some cases this has been caused by fibrous tissue, and in other cases there is an apparent obliteration due to precipitation of albuminous material similar to that in the subcapsular space. In some of the lobes the capillaries are gorged with red cells. There has been formed a new epithelial capsule around the tuft, which is continuous across the intercapillary loops. In one place, at the tip of the tuft, next to the neck of the tubule, this epithelium seems to be almost of the columnar type; in most of its extent, however, it is formed of cells of a thick, squamous character. In some of the capillaries, especially the main intralobular branches, there is hypertrophy of endothelium, but apparently in no case does it completely close the capillary. There are no histologic reasons apparent why a fairly good supply of blood should not pass through this glomerulus. There are no adhesions between the glomerular tuft and the capsule. The epithelium of the capsule is fairly normal.

The greatest diameter of the glomerulus as measured by Dr. Turley from the original sections is 275 microns from capsule to capsule. As measured from the model, the maximum diameter at right angles to the direction aforementioned is 235 microns, and at right angles to the latter measurement the diameter is 170 microns. Compared with the normal, the glomerulus is obviously considerably enlarged.

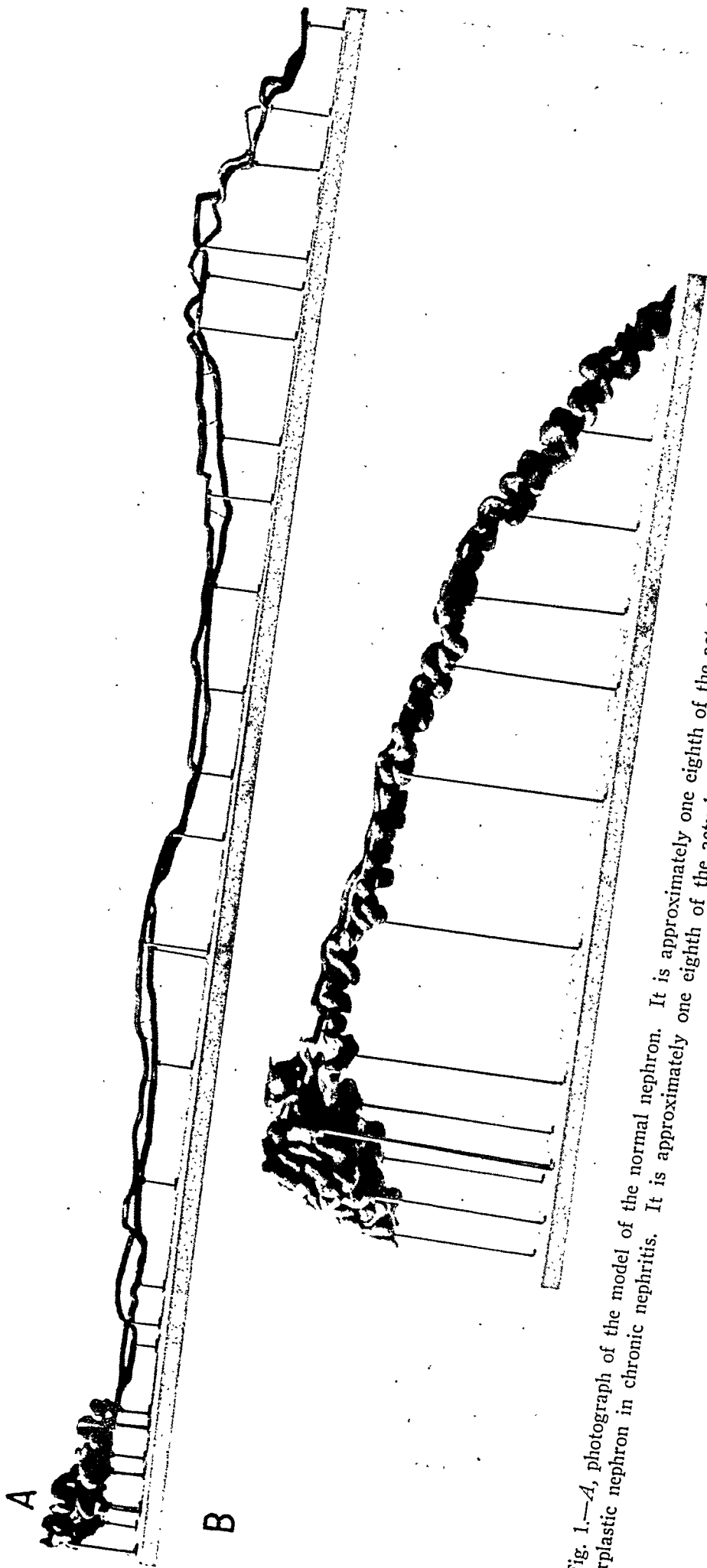
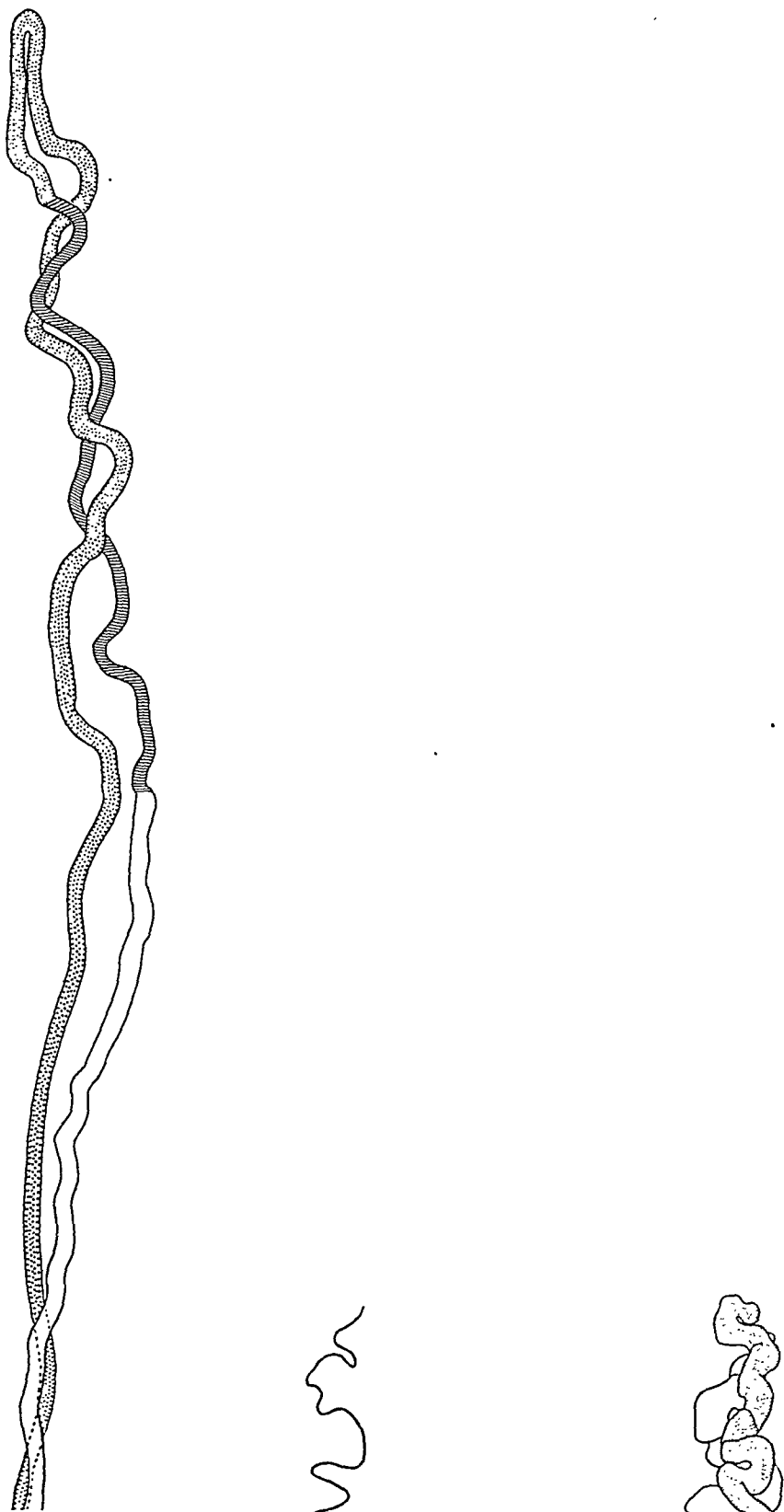


Fig. 1.—*A*, photograph of the model of the normal nephron. It is approximately one eighth of the actual size of the model. *B*, photograph of the model of the hyperplastic nephron in chronic nephritis. It is approximately one eighth of the actual size of the model.

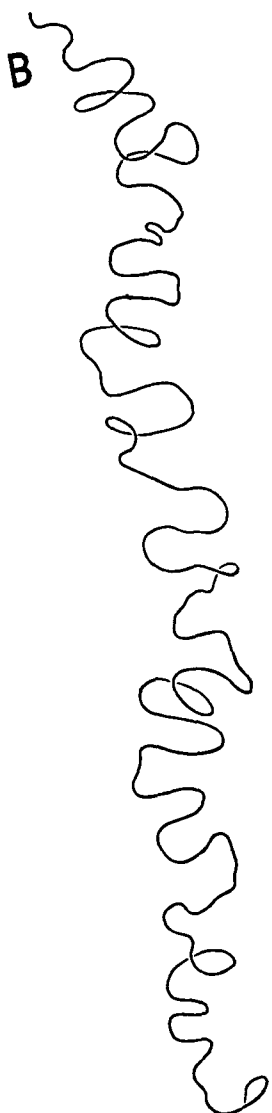
colored red. $\times 50$.
 brown; the point of entrance of the afferent vessel is shown as a small projection, tubule are colored green, the thin segment yellow and the entire distal tubule the distal tubule is dotted. In the original model the glomerulus and entire proximal (recta) are represented with simple lines. The thin segment is cross lined, and mass of the convoluted portion) and the proximal tubule (pars convoluta and pars 60 degrees to the horizontal. The glomerulus (to the left of and below the main C, drawing of the model of the normal nephron as viewed at an angle of about drawing is oriented the same as A. $\times 50$.

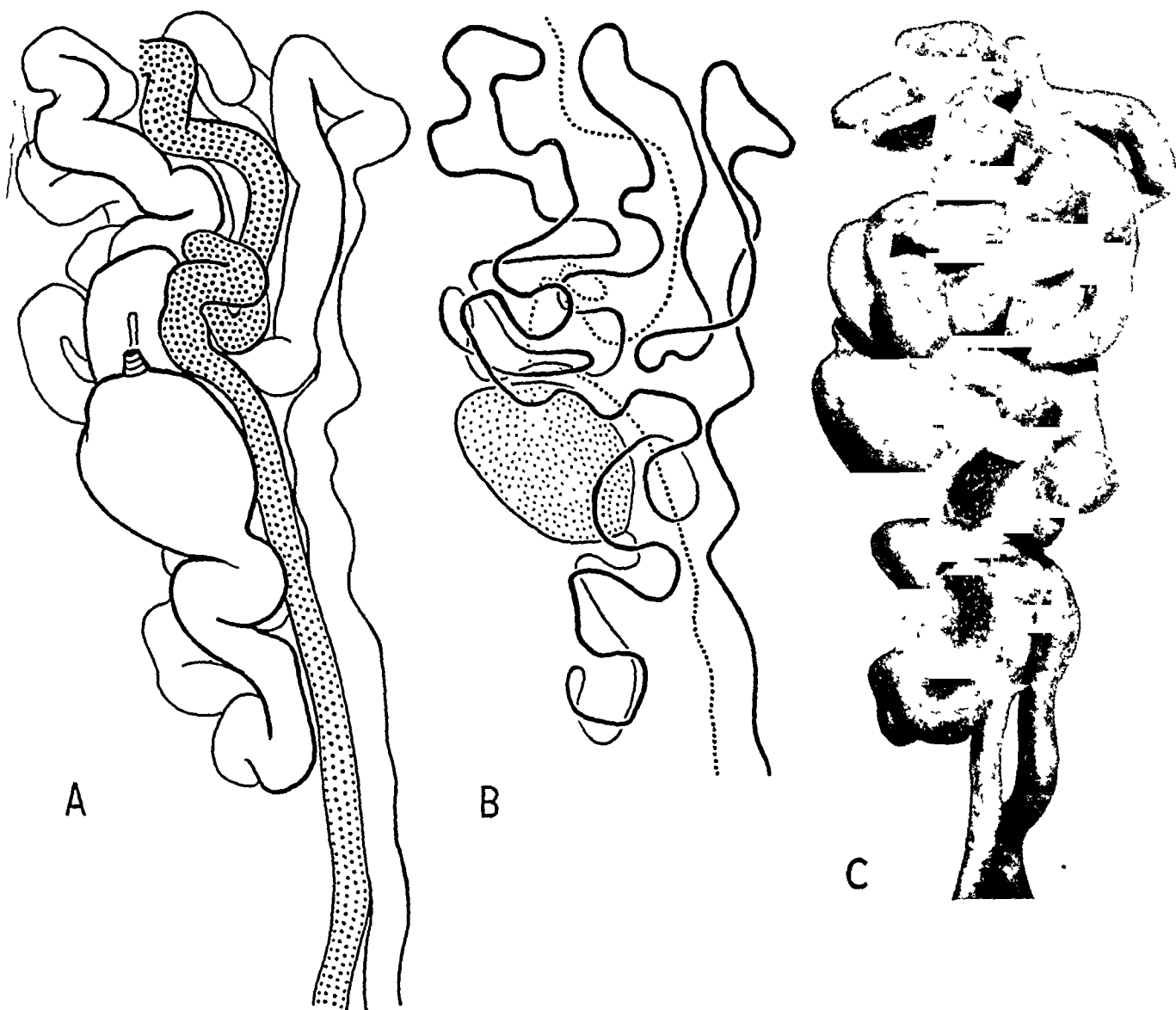
of the proximal tubule in the hyperplastic nephron in chronic nephritis. This B, a "wire" diagram, analyzing the course of the markedly irregular pars recta right of the glomerulus) is cross lined. $\times 50$.

dotted, and the cut surface of the distal tubule (which is located above and to the convoluta and pars recta) are represented by simple lines. The distal tubule is looking vertically down on the model. The glomerulus and proximal tubule (pars Fig. 2.—A, drawing of the model of the hyperplastic nephron in chronic nephritis,



B





EXPLANATION OF FIGURE 3

A, Drawing of the inferior aspect of the convoluted portions of the normal nephron to show the first portion of the proximal convoluted tubule as it leaves the glomerulus and the course of the distal convoluted tubule (dotted). The short projection (cross lined) at the superior pole of the glomerulus represents the point of entrance of the afferent vessel. This view of the model was obtained by the use of a mirror, and is at somewhat of an angle from the horizontal plane. $\times 100$.

B, a "wire" diagram of the convoluted portions of the normal nephron, analyzing the course of the proximal (solid line) and distal (dotted line) convoluted tubules. The oval dotted area to the left represents the glomerulus. The diagram is oriented as in C for comparison. Where one portion of the proximal convoluted tubule passes under another portion, this is indicated by a break in the black line. The distal convoluted tubule is all located on the inferior aspect of the model (see A) and at no point intertwines with the convolutions of the proximal tubule. $\times 100$.

C, photograph of the convoluted portion of the normal nephron. The glomerulus is located to the left of the center, and the point of entrance of the afferent vessel is shown as a small projection from the superior pole of the glomerulus. At the bottom of the picture the beginning of the pars recta of the proximal tubule is seen to the right, and the upper portion of the pars recta of the distal tubule is seen to the left. Except for these short portions of the descending and ascending limbs and of the glomerulus, the entire tubular mass illustrated belongs to the proximal convoluted tubule. The photograph shows the view obtained by looking directly down on the model, and B is similarly oriented for comparison. $\times 100$.

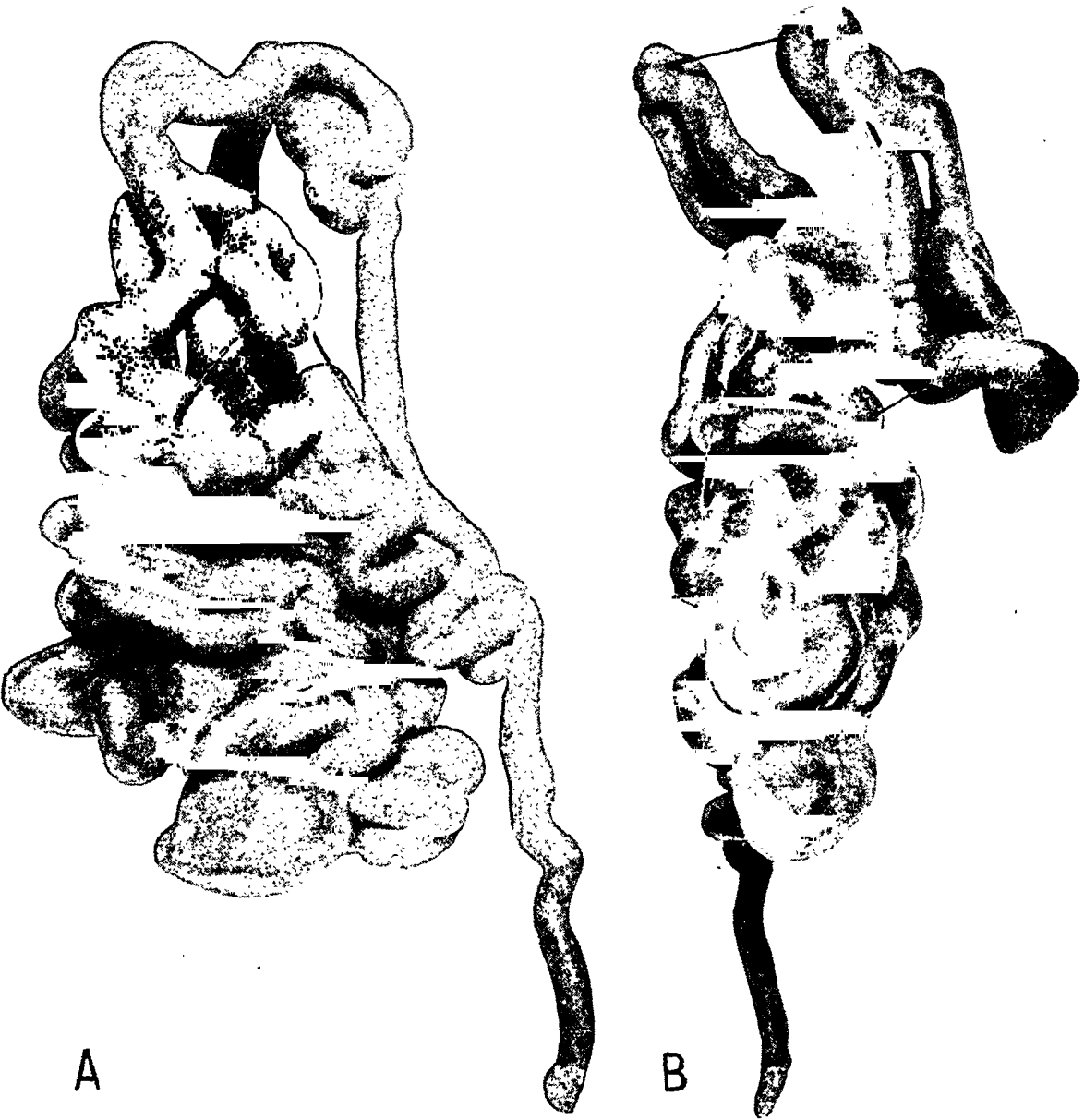


Fig. 4.—*A*, photograph of the superior aspect of the model of the glomerulus (seen at the inferior pole of the main mass) and the proximal convoluted tubule in acromegaly. The upper portion of the pars recta of the proximal tubule is shown below and to the right. $\times 100$.

B, photograph of the model of the glomerulus and proximal convoluted tubule in acromegaly, viewed from the side (at right angles to *A*). $\times 100$.

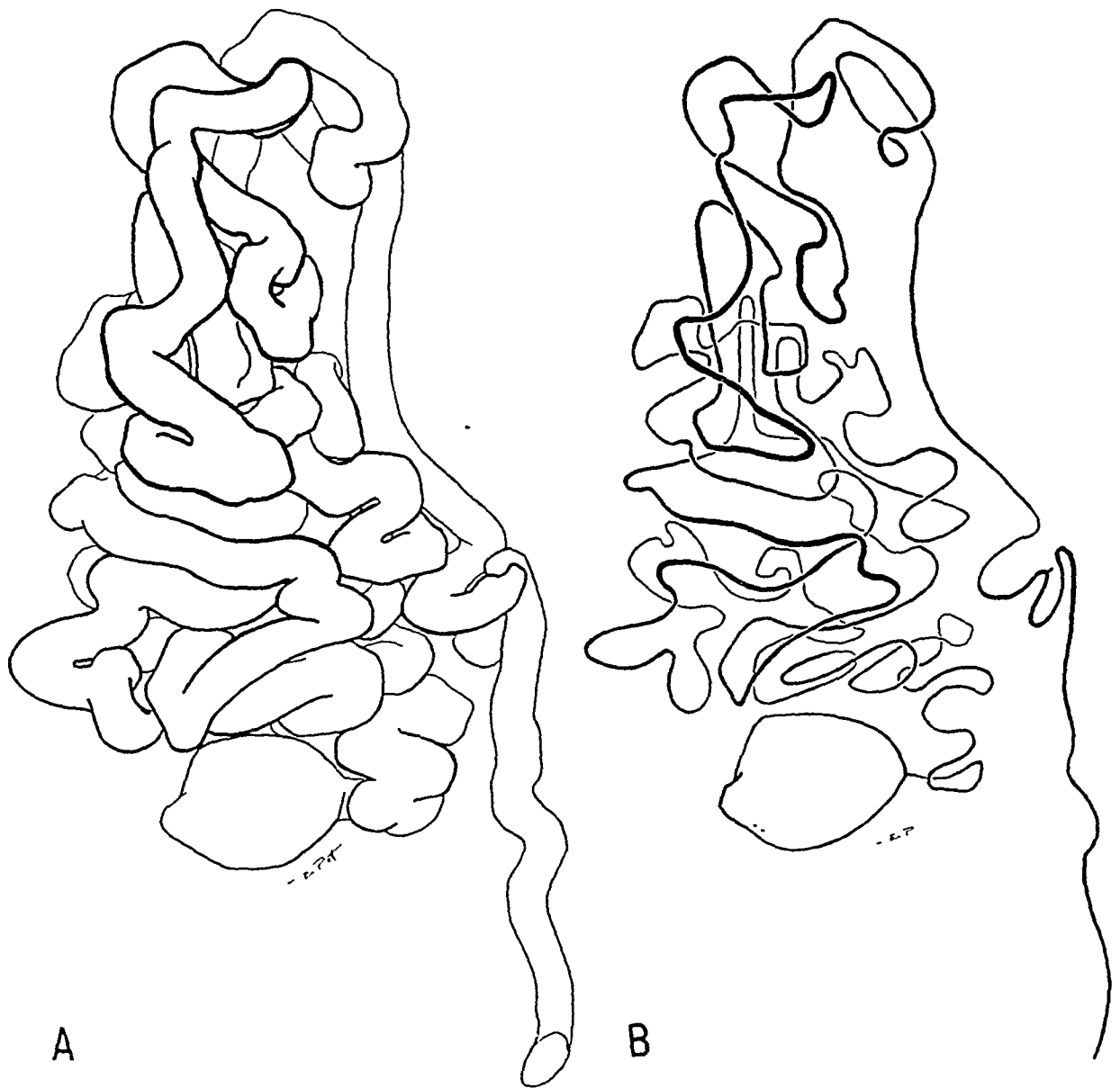
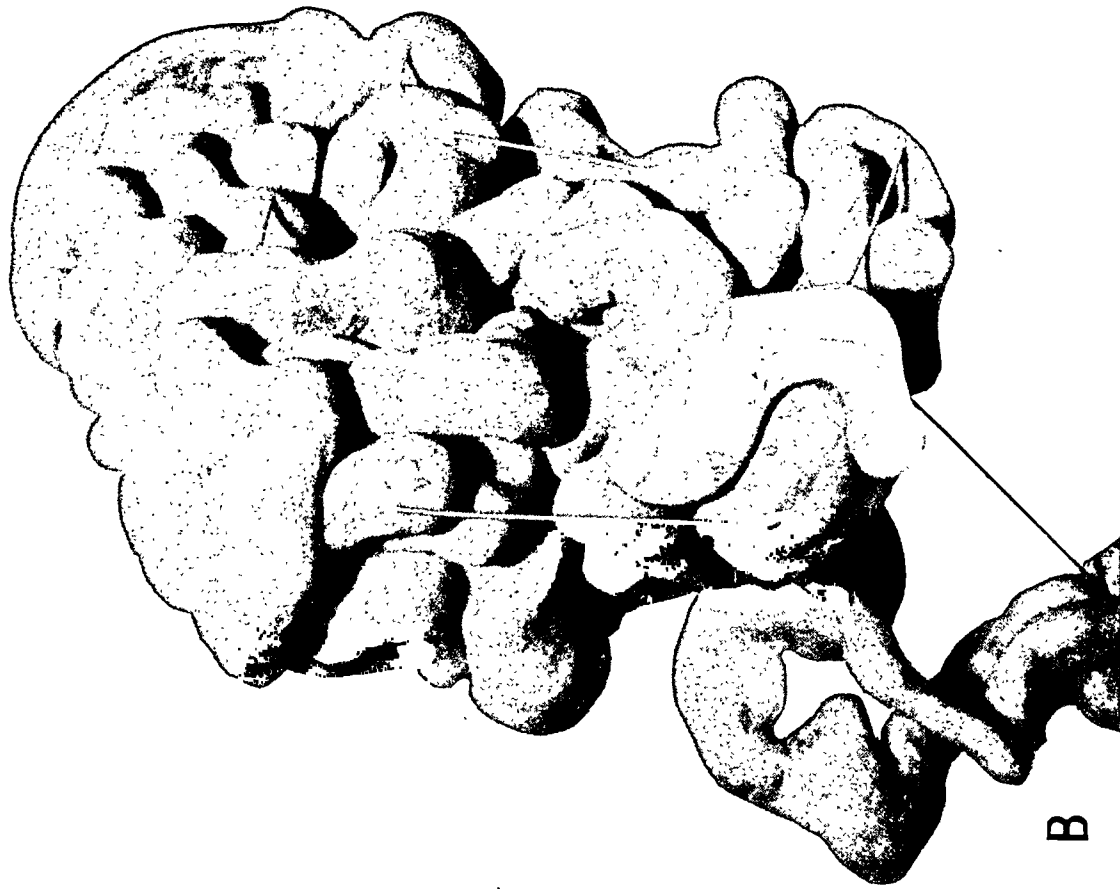


Fig. 5.—*A*, drawing of the model of the glomerulus and proximal convoluted tubule in acromegaly, oriented as in figure 4 *A*. $\times 100$.

B, a "wire" drawing of the proximal convoluted tubule in acromegaly, analyzing the course of the convolutions. The figure is oriented as in figures 4 *A* and 5 *A*. The oval dotted area represents the glomerulus. Where one portion of the proximal convoluted tubule passes under another portion, this is indicated by a break in the black line. The linear representation of the pars convoluta shows a short projection near the beginning of the pars recta (to the right, below the center). This represents a small diverticulum of the tubule at this point; this diverticulum can be seen in figure 4 *A* on careful examination, and it is illustrated in *A* in the present figure. $\times 100$.



A



B

Fig. 6.—*A*, photograph of the enlarged proximal convoluted tubule in the model of the hyperplastic nephron in chronic nephritis. This is a side view, taken at an angle of about 45 degrees. It is a view of the side opposite that shown in figure 1*B*. The entire mass shown is the proximal tubule with the exception of a short stretch of the distal tubule (at the lower right hand corner; compare *B*). The glomerulus and its urinary pole can be seen behind and to the right of the cut surface of the distal tubule. $\times 100$. *B*, photograph of the upper portion of the model of the hyperplastic nephron in chronic nephritis, looking directly down on the model. The irregular light gray area represents the cut surface of the distal tubule (compare figures 2*A* and 7*B*, which are oriented in the same way). The glomerulus is located to the left of, and behind, this cut surface. In the lower left hand corner a short stretch of the distal tubule can be seen overlying the proximal tubule (larger in diameter, course very irregular). $\times 100$.

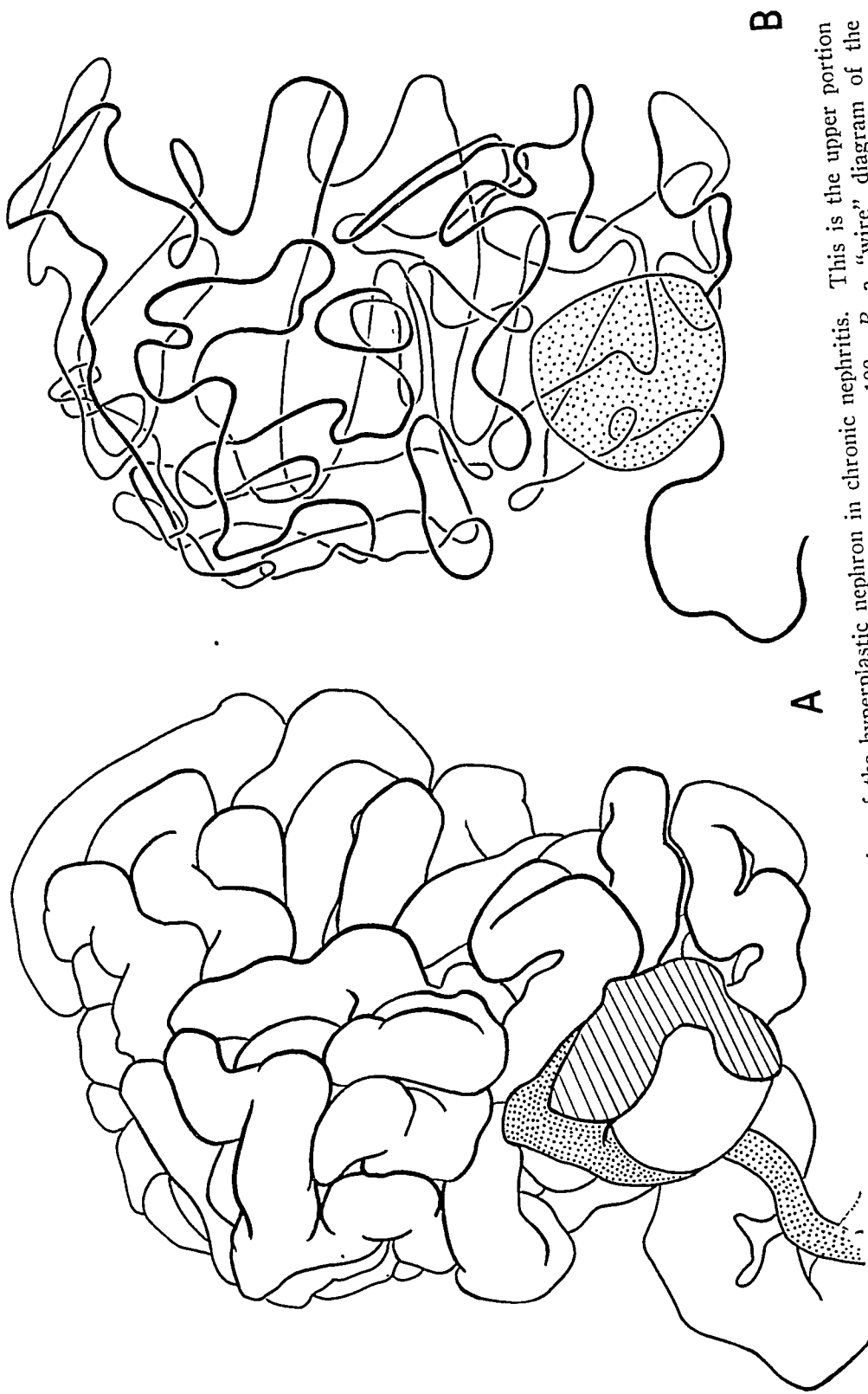
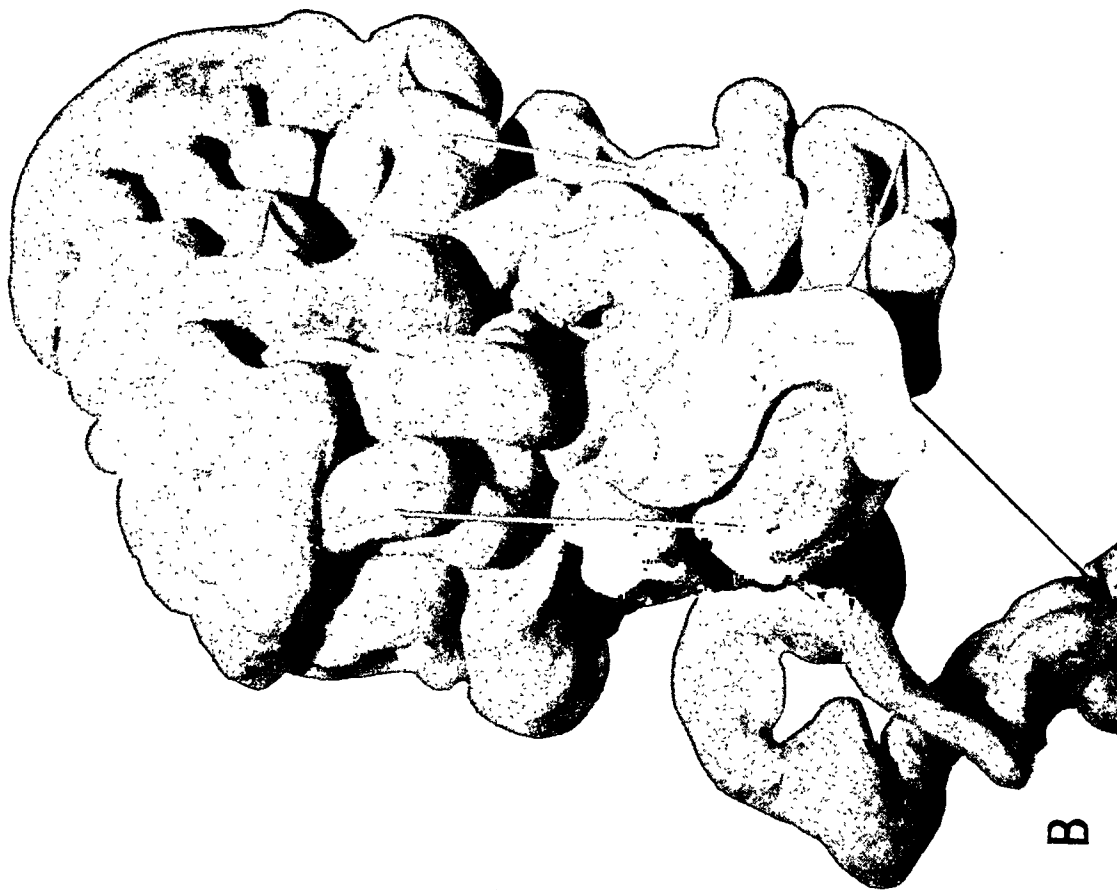


Fig. 7.—*A*, drawing of the convoluted portion of the hyperplastic nephron in chronic nephritis. This is the upper portion of figure 2*A*, reproduced at larger magnification for direct comparison with *B*. $\times 100$. *B*, a "wire" diagram of the proximal convoluted tubule in the hyperplastic nephron in chronic nephritis, oriented the same as in *A*. The dotted area represents the glomerulus. Where one portion of the proximal convoluted tubule passes under another portion, this is indicated by a break in the black line. $\times 100$.



A



B

Fig. 6.—*A*, photograph of the enlarged proximal convoluted tubule in the model of the hyperplastic nephron in chronic nephritis. This is a side view, taken at an angle of about 45 degrees. It is a view of the side opposite that shown in figure 1 *B*. The entire mass shown is the proximal tubule with the exception of a short stretch of the distal tubule (at the lower right hand corner; compare *B*). The glomerulus and its urinary pole can be seen behind and to the right of the cut surface of the distal tubule. $\times 100$. *B*, photograph of the upper portion of the model of the hyperplastic nephron in chronic nephritis, looking directly down on the model. The irregular light gray area represents the cut surface of the distal tubule (compare figures 2 *A* and 7 *B*, which are oriented in the same way). The glomerulus is located to the left of, and behind, this cut surface. In the lower left hand corner a short stretch of the distal tubule can be seen overlying the proximal tubule (larger in diameter, course very irregular). $\times 100$.

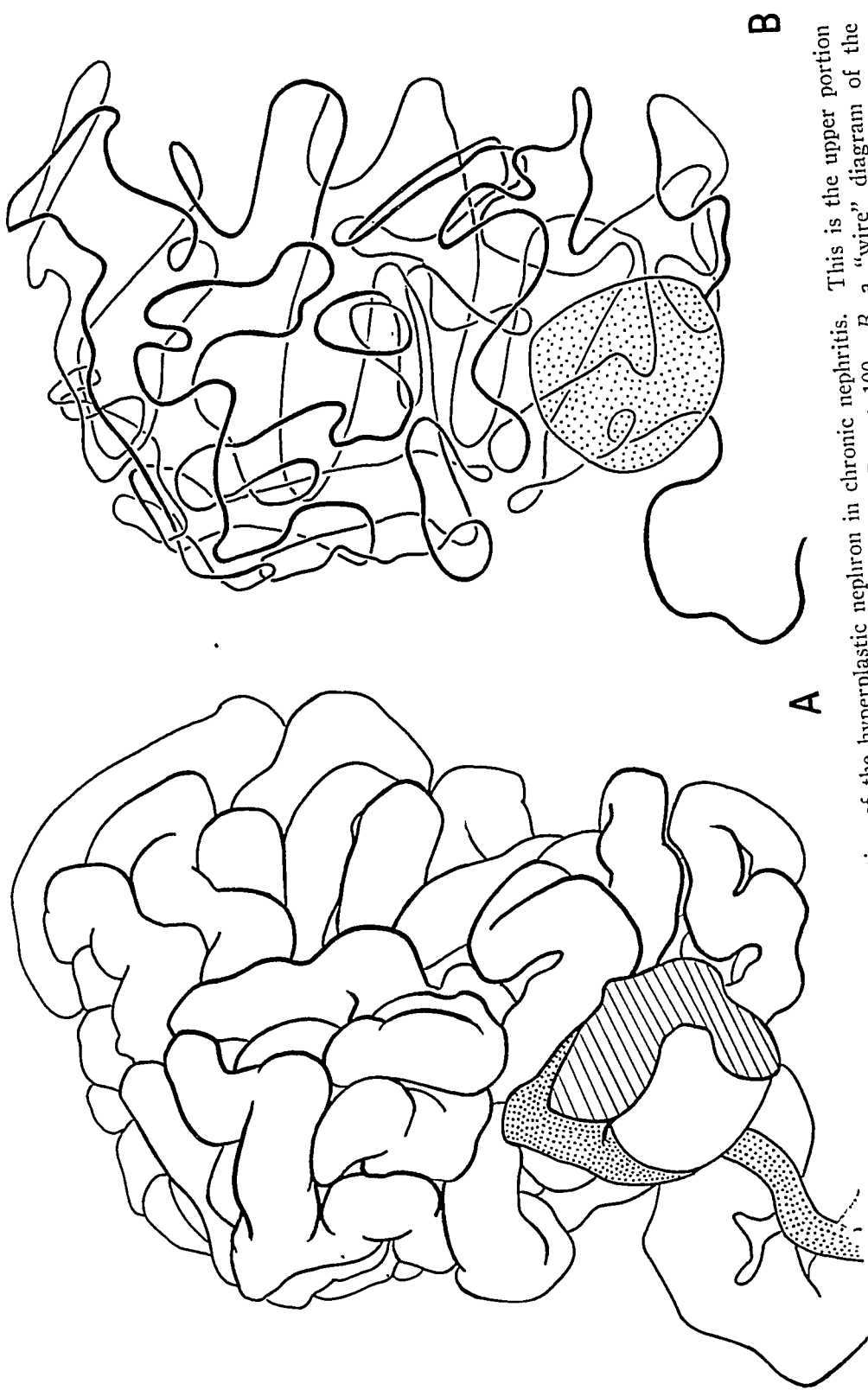


Fig. 7.—*A*, drawing of the convoluted portion of the hyperplastic nephron in chronic nephritis. This is the upper portion of figure 2*A*, reproduced at larger magnification for direct comparison with *B*. $\times 100$. *B*, a "wire" diagram of the proximal convoluted tubule in the hyperplastic nephron in chronic nephritis, oriented the same as in *A*. The dotted area represents the glomerulus. Where one portion of the proximal convoluted tubule passes under another portion, this is indicated by a break in the black line. $\times 100$.

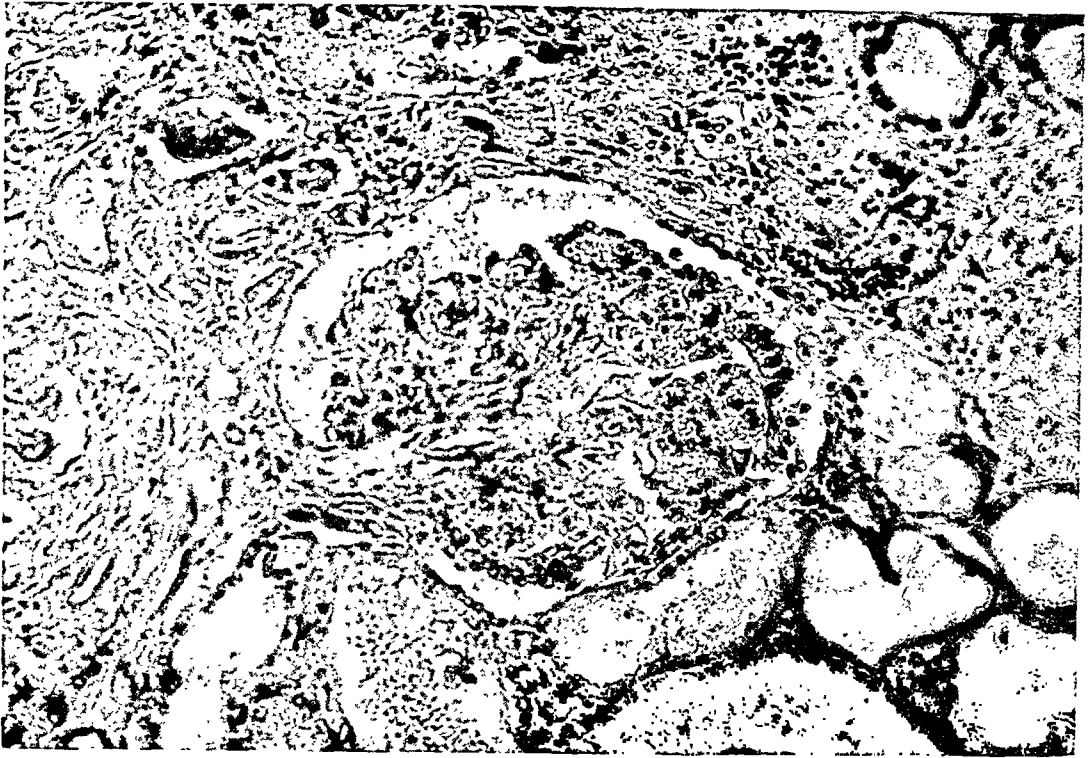


Fig. 8.—Photomicrograph of the glomerulus of the hyperplastic nephron modeled by Turley. The urinary pole is to the right, and the flattened character of the epithelium of the first portion of the proximal tubule is well shown. The sectioned tubules beneath and to the right of the glomerulus are portions of the hyperplastic proximal tubule of the same nephron. The photograph was supplied by Dr. Turley.

COMMENT

The Method of Reconstruction.—Turley's description of his method of reconstruction has been quoted in the foregoing part of this article in detail because of Peter's⁹ statements concerning the extreme difficulty of reconstructing adult mammalian urinary tubules. For a discussion of the use of orientation lines (Ritzer lines, etc.) in reconstruction, it is sufficient here to refer to the well known paper of Born and Peter.¹³ In Peter's modeling of the adult human nephron (see comment later) the serial sections were provided with such base or orientation lines, supplied by coating one surface of the paraffin block with "nubian blacking." These lines he considered indispensable, stating that in his opinion it is impossible to make an accurate reconstruction of such a complicated tubule without them. Nevertheless, from the recent experiences of Oliver and Lund,⁴ from my own experience in modeling the nephrons of lower vertebrates¹⁴ and from the

13. Born, G., and Peter, K.: *Ztschr. f. wissenschaft. Mikr.* **15**:31, 1898.

14. Grafflin, A. L.: *Anat. Rec.* **68**:287, 1937.

general experience of other workers in modeling various tissues, I am entirely convinced of the adequacy of the method Turley employed, and I believe that his models can be accepted as wholly accurate plastic representations of the nephrons which he studied.

The Normal Nephron.—In addition to Turley's model, only one other extensive plastic reconstruction of the normal adult human nephron has ever been produced. This is the well known model made by Peter,⁹ which is less complete than Turley's in that it does not include the loop of Henle, but more complete in that it includes (a) the entire junctional tubule and a portion of the collecting duct into which tubule drains, (b) the afferent vessel and a portion of the interlobular¹⁵ vessel from which the afferent vessel arises and (c) a portion of the efferent vessel. Peter's excellent illustrations should be compared in the original with those given here, since the two models supplement each other so excellently. The present illustrations have for the most part been reproduced at the same magnification as in Peter's article ($\times 100$) to render such a comparison easier and more profitable. At the same time, drawings of the nephritic and normal nephron have been reproduced (fig. 2A and C) at the same magnification as those of Oliver and Lund ($\times 50$) for purposes of comparison.

A consideration of Turley's model shows that it probably represents a fairly typical "short loop" human nephron. The glomerulus¹⁶ and proximal convoluted tubule of the nephron lay "about midway between the capsule and the arcuate vessels" (see quoted paragraph, page 694); the thin segment is quite short (about 1.5 mm.), and the bend in the loop of Henle occurs in the distal tubule. As Peter⁹ demonstrated, "short loop" nephrons are at least seven times as numerous as "long loop" nephrons. The glomeruli of the latter lie deep in the cortex, and the bend in the loop of Henle regularly occurs in the course of the thin segment, which is usually quite long. It is to be emphasized that in an occasional human nephron the thin segment is entirely absent, the proximal tubule continuing directly in the distal tubule.¹⁷ The glomer-

15. This is the usual designation for these vessels. It is in keeping with the concept of a structural unit of the kidney built about the collecting ducts, in the sense of H. F. Traut (*The Structural Unit of the Human Kidney*, *Contrib. Embryol.* **15**:103, 1923). W. von Möllendorff (*Der Exkretionsapparat*, in *Handbuch der mikroskopischen Anatomie des Menschen*, Berlin, Julius Springer, 1930, vol. 7, pt. 1, p. 1), on the other hand, sharply disagrees with this usage; he supports instead the concept of a vascular unit of the kidney, built about the so-called interlobular vessels, which he designates "intralobular."

16. The size of the glomerulus as measured from the model is approximately 235 by 163 microns—almost identical with the glomerulus in the model of the acromegalic nephron.

17. Peter, K.: *Zum feineren Bau der menschlichen Niere*, in *Untersuchungen über Bau und Entwicklung der Niere*, Jena, Gustav Fischer, 1927, no. 2, p. 449; footnote 9.

uli of such nephrons lie peripherally in the cortex, and the loop is confined to the cortex or extends only a short distance into the medulla.

In both the Peter and the Turley model the ascending limb of the loop of Henle "returns" to apply itself to the glomerulus of the same nephron, lying in close apposition to the vascular pole. This relationship was first shown by Golgi¹⁸ and was confirmed and shown to be invariable—in man and all other mammals investigated—by Hamburger¹⁹ and Peter.⁹ The latter investigators emphasized the close relationship of the distal tubule to the vas efferens. That the essential relationship is, rather, to the vas afferens, Turley was apparently the first to appreciate. In his thesis he strongly insisted on this relationship and stated that "anyone can be sure that he has the ascending limb of the loop of the tubule to any glomerulus by finding the afferent artery to the glomerulus and taking the ascending tubule found there without following out the whole system."

This dictum of Turley's has been strongly reenforced by the extensive study of Michailovitch.²⁰ As is now well known, the epithelium of the distal tubule shows a striking dense accumulation of nuclei—the "macula densa," so named by the late Prof. K. W. Zimmermann—at the point of apposition to the vascular pole of the glomerulus. In a reinvestigation of the problem of the exact relationship of the ascending limb to the glomerulus, Michailovitch made a careful study of the "macula densa" in twelve different species: man, baboon, rhesus monkey, horse, cat, dog, pig, ox, rabbit, guinea pig, mouse and rat. His conclusions may be briefly summarized: In all animals investigated the ascending limb is in direct apposition to the vascular pole of the glomerulus and, more specifically, is intimately attached to the vas afferens near its point of entrance into the glomerulus. That this intimate association with the vas afferens is the important and essential relationship is shown by the fact that one finds here, and only here, the formation of the "macula densa" in the tubular wall. The "macula densa" is developed only on one side of the tubule—the side adjacent to the vas afferens—and is of variable length, with a maximal observed length in man of 66.6 microns. The ascending limb usually crosses the vas afferens at the point of contact but can lie lengthwise along it (observed in the dog and the pig). The close packing of the nuclei was found to be less marked in the cat than in any of the other animals investigated.

18. Golgi, C.: *Rendic. d. r. Accad. d. lincei, Cl. di sc. fis., mat. e nat.* 5:334, 1889.

19. Hamburger, O.: *Arch. f. Anat. u. Entwcklungsgesch.*, 1890, supp., p. 15.

20. Michailovitch, V.: *Wie verhält sich der aufsteigende Schenkel der Henle'schen Schleife zum corpusculum renis?* Inaug. Dissert., Berne, Switzerland, 1919 (unpublished; consulted in the original in Berne).

Djokic²¹ observed the "macula densa" in the bear and confirmed its association with the vas afferens, and Belosavitch²² made the same observation for the llama. Von Möllendorff,²³ in his recent treatise on the kidney, fully accepts the conclusion of Michailovitch (and so of Turley, though the latter's work was unknown to him) that the essential relationship of the ascending limb is with the vas afferens.

In view of the invariability of this association of the ascending limb with the vascular pole, and in particular with the vas afferens, of the glomerulus of the same nephron, it seems that one need never again experience the difficulties encountered by Oliver and Lund.⁴ In describing the technic of making their reconstructions of the atrophic and hypertrophic nephrons in chronic nephritis, they wrote as follows:

The identification of the distal convolution, including the connecting tubule and the collecting tubule, offered a somewhat more difficult problem. No certain point of departure was available, since it is impossible to trace the tubule through Henle's loop . . . and then back to the region of the glomerulus. Cross-sections of what appeared to be the corresponding distal convolutions were therefore chosen and followed through the drawings. The fifth attempt proved successful, as was shown by the inclusion of the selected tubule within the loops of the proximal convolutions.

In concluding the comment on this aspect of the problem, it is well to point out that an intimate apposition of the future distal tubule to the glomerulus dates from the very beginning of the embryologic development of the nephron and is never lost in the subsequent development of the loop of Henle.

Although it is not specifically stated in his thesis, Dr. Turley has informed me that the model of the normal nephron includes the entire distal convoluted tubule and is interrupted at the point where the latter joins the junctional tubule.

The Glomerulus and Proximal Convoluted Tubule in Acromegaly.—As stated, Turley's model of the glomerulus and proximal convoluted tubule in acromegaly is entirely unique. That the disease is usually characterized by "a general splanchnomegaly of the viscera which is disproportionate to the general enlargement of the body" and that the liver and kidneys are particularly enlarged have been emphasized by Cushing and Davidoff.²⁴ They gave the average combined weight

21. Djokic, A. M.: Zur Histologie der Bärenniere, Inaug. Dissert., Berne, Switzerland, 1919 (unpublished; consulted in the original in Berne).

22. Belosavitch, N.: Ueber den Bau der Lamaniere, Inaug. Dissert., Berne, Switzerland, 1919 (unpublished; consulted in the original in Berne).

23. von Möllendorff, W.: Der Exkretionsapparat, in Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1930, vol. 7, pt. 1, p. 1.

24. Cushing, H., and Davidoff, L. M.: The Pathological Findings in Four Autopsied Cases of Acromegaly with a Discussion of Their Significance, Monograph 22, Rockefeller Institute for Medical Research, 1927.

of the kidneys in 25 cases as 576 Gm., with a maximum of 1,170 Gm. They further stated that in their own cases "the huge organs on the whole have been well within histologically normal limits."

It will be recalled that in the case in which Turley made his reconstruction the weight of the kidneys was approximately twice the normal and that no abnormal histologic change was observed. The essential fact contributed by the model is that in the case of acromegaly studied by Turley the pars convoluta of the proximal tubule of the particular nephron reconstructed had undergone a striking increase in length but not in diameter and that the increase in length was roughly proportional to the enlargement of the kidney as a whole (approximately twice normal). The size of the glomerulus as measured from the model is approximately 235 by 160 microns, which is within the normal range. In 2 of 4 cases of acromegaly reported by Cushing and Davidoff, on the other hand, as they definitely stated, the glomeruli were enlarged (those in case I being "large"; those in case III, "distinctly enlarged"—average diameter, 250 microns), and Schultze and Fischer²⁵ reported a marked enlargement of the glomeruli (diameter, from two to three times normal) in a case of theirs. With respect to the tubules, Cushing and Davidoff stated that "the tubules, particularly the convoluted, are definitely dilated" (their case I) and again that "all the tubules are large, the convoluted average 90 μ in diameter as compared with a normal of 70 μ " (their case III). To my knowledge, no measurements of the length of the renal tubules in cases of acromegaly have ever been reported.

Obviously, a great deal more work must be done in order to clarify the factors underlying the enlargement of the kidney in acromegaly. Turley's model is a distinct contribution but represents, after all, only a single glomerulus and proximal convoluted tubule in a single case. Accurate measurements of large numbers of complete nephrons and of their various subdivisions are clearly desirable; for such a study the maceration technic, supplemented by examination of histologic sections, would be the method of choice.²⁶

The Hyperplastic Nephron in Chronic Nephritis.—There is available only one other model of the hyperplastic nephron in chronic nephritis with which Turley's model can be compared, namely, that of Oliver and

25. Schultze, F., and Fischer, B.: *Mitt. a. d. Grenzgeb. d. Med. u. Chir.* **24**: 607, 1912.

26. The maceration technic has been applied by Peters (Peters, E.: *Arch. f. Zellforsch. u. mikr. Anat.* **8**:63, 1928) to kidneys showing compensatory hypertrophy after unilateral nephrectomy. The study was carried out on white mice. While there was some increase in glomerular size, the most striking finding was an increase in the length of the proximal tubule; unfortunately, the measurements did not include the remaining segments of the nephron.

Lund.⁴ Their model is less complete than Turley's in that it does not include any significant portion of the ascending and descending limbs of Henle's loop, but more complete in that it includes (*a*) the entire distal convoluted tubule, the junctional tubule and a portion of the collecting duct into which the latter drains, (*b*) the afferent vessel and a portion of the interlobular vessel from which the afferent vessel arises, and (*c*) a portion of the efferent vessel. The two models supplement each other well, and the Oliver and Lund illustrations should be carefully compared with those given here. The outstanding feature of the hyperplastic nephron in chronic nephritis is clearly a massive enlargement of the proximal tubule. The remaining segments of the nephron share not at all in this hypertrophy or do so to only a very minor extent. Though the Oliver and Lund model includes only the pars convoluta of the proximal tubule, Oliver and Luey⁵ subsequently demonstrated, by maceration studies, that the enlargement involves as well the entire pars recta, which shows a remarkably tortuous course, being folded back and forth on itself. The strikingly altered appearance of the pars recta of the proximal tubule is well shown in Turley's model, although it is to be emphasized that the model is not complete in this respect, i. e., stops short of the transition to the thin segment.

The status of the glomeruli in the two reconstructed hyperplastic nephrons is interesting. The histologic description of the glomerulus in Turley's nephron leaves no doubt that the functional capacity of the glomerulus must have been markedly reduced. In the nephron of Oliver and Lund the glomerulus was definitely increased in size, but "the great majority of the tuft capillaries and the greater part of Bowman's space were obliterated, and the original free surfaces, which must have been of great importance in glomerular activity, were greatly reduced." In other words, from the functional standpoint their hyperplastic nephron was essentially "aglomerular." From the data available, such functionally aglomerular nephrons must be of very frequent occurrence. Furthermore, Oliver and Luey²⁷ were able to demonstrate the not infrequent occurrence of truly aglomerular nephrons, in which all physical connection with the glomerulus had been lost by a complete interruption of the nephron at some point in the proximal tubule. Yet these functionally aglomerular and truly aglomerular nephrons must be considered, from the morphologic point of view at least, as capable of considerable functional activity.

As is now well known, aglomerular kidneys occur in many species of teleostean fishes, a fact which has been of considerable interest in studies of the physiology of the vertebrate nephron (for a broad state-

27. Oliver, J., and Luey, A. S.: *Arch. Path.* **19**:1, 1935.

ment of the problem see Marshall²⁸). Important for present consideration are the following facts: (1) The naturally occurring aglomerular nephron in the teleost consists solely²⁹ of a segment provided with a brush border, i. e., the homologue of the proximal tubule of the higher vertebrates;¹⁴ such aglomerular nephrons show a marked capacity for tubular excretion.³⁰ (2) In glomerular teleosts in which the nephron exhibits only the proximal tubule, the latter segment likewise shows considerable tubular excretion, and when such nephrons are rendered functionally aglomerular by the injection of repeated large doses of phlorhizin the tubular excretory capacity of the proximal tubule can be quite marked.³⁰

The interest of these facts in connection with the findings of Oliver and his associates and of Turley in human nephritis is obvious. In the hyperplastic nephrons which they have studied and which may be functionally or truly aglomerular, it is only the proximal tubule which shows massive enlargement, the remaining segments of the nephron remaining essentially normal. The proximal tubule is the only subdivision invariably present in the vertebrate nephron, and it appears to be the fundamental segment of the renal tubule. In the lower vertebrates the excretory capacity of the proximal tubule—whether a glomerulus is present or not—has been adequately demonstrated. In man, on the other hand, tubular excretion—under normal conditions—apparently plays a very minor role in the formation of urine.³¹ Since in advanced chronic nephritis the burden of renal function is apparently borne in large measure by the hyperplastic nephrons, since massive enlargement is exhibited only by the proximal tubules and since these hyperplastic nephrons may be functionally or truly aglomerular, it appears reasonable to assume that such hyperplastic nephrons are the site of marked tubular excretion and that this tubular excretion—primarily at least—is taking place in the enormously hyperplastic proximal tubule. The implication is that the proximal tubule, primarily reabsorptive in man under normal conditions, is able, under certain pathologic conditions, to develop in marked degree the capacity for tubular excretion which characterizes this segment in lower vertebrates. Important in this connection is the following general conclusion of Marshall:²⁸ “In the human kidney, where filtration-reabsorption appears under ordinary conditions to play

28. Marshall, E. K., Jr.: *Physiol. Rev.* **14**:133, 1934.

29. There is likewise present a short junctional tubule such as characterizes all vertebrate nephrons; this junctional tubule is usually regarded merely as a conduit and will be disregarded in the present discussion.

30. Marshall, E. K., Jr., and Graffin, A. L.: *J. Cell. & Comp. Physiol.* **1**:161, 1932.

31. Smith, H. W.: *The Physiology of the Kidney*, New York, Oxford University Press, 1937.

a predominant role for the normal urinary constituents, it is possible that under certain pathological conditions the more primitive secretory process in the tubule may be of major importance."

MacNider,³² in his studies of experimental chronic nephritis from uranium nitrate in dogs, pointed out that in areas of the kidney exhibiting complete obliteration of the glomeruli "a modified type of (proximal) convoluted tubule epithelium has been preserved"; he went on to state that "such areas resemble the normal structure first described by Marshall³³ for the aglomerular toadfish, *Opsanus tau*." On the basis of his observations MacNider suggested the possibility "that an organ, the seat of processes of degeneration followed by repair, may as a result of the latter process revert back to a type of structure normal for a remote ancestral form."

The occurrence and probable significance of the aglomerular nephrons in human chronic nephritis were extensively discussed by Oliver and Luey,²⁷ whose treatment of the problem should be consulted. These authors and later Loomis⁶ likewise discussed in detail the problems associated with the maintenance of an adequate blood supply to the diseased kidney. In the latter connection I should like to call attention here to the recent and very important contribution of Spanner,³⁴ who was able to demonstrate the occurrence of arteriovenous anastomoses in considerable numbers in the normal human kidney.

Some years ago I was faced with the paradox of a kidney which exhibited numerous glomerular structures anatomically, yet behaved functionally as a completely aglomerular organ.³⁵ This observation was made in an old specimen of *Myoxocephalus scorpius* (a marine teleost), and it became necessary to attempt to reconcile the anatomic with the physiologic observations, particularly since younger specimens of the same species gave some evidence of glomerular function, and since the kidney of a closely related species, *Myoxocephalus octodecimspinosus*, regularly showed marked glomerular function.³⁶ Histologic study of the kidney in a series of specimens of *M. scorpius*, varying in weight from 85 to 1,006 Gm., satisfactorily explained the paradox in the very old specimen and led to the following conclusions:

The glomeruli have been rendered incompetent by degenerative changes affecting both the vascular tufts and the neck segments, so that it is practically impossible to find a single glomerulus which on anatomical grounds can be considered functional. In young fish of the same species there can be found adequate anatomical

32. MacNider, W. deB.: *Proc. Soc. Exper. Biol. & Med.* **31**:293, 1933.

33. Marshall, E. K., Jr.: *Bull. Johns Hopkins Hosp.* **45**:95, 1929.

34. Spanner, R., in *Verhandlungen der anatomischer Gesellschaft, Anat. Anz.* (supp.) **85**:81, 1938.

35. Grafflin, A. L.: *Anat. Rec.* **57**:59, 1933.

36. Grafflin, A. L.: *Anat. Rec.* **68**:145, 1937. Marshall and Grafflin.³⁰

basis for the varying, but low, glomerular function which can be demonstrated physiologically. However, considerable degeneration is already present in the youngest specimens examined, and these changes become steadily more prominent with increasing age. . . . This degeneration is interpreted as a physiological involution rather than as a pathological process.

Despite the widespread obliteration of the glomerulus and of its associated neck segment, the remainder of the nephron, consisting solely of the proximal tubule, almost uniformly persists. Furthermore, the persistent aglomerular nephrons are entirely normal in appearance, and in no single instance do they exhibit the marked hyperplastic changes observed in the proximal tubule in human nephritis.

It will be recalled that in his thesis Turley made the following statement concerning the hyperplastic nephrons in chronic nephritis: "The epithelium of the tubule as it leaves the glomerulus is reduced to a squamous form, and this change extends for a considerable distance along the tubule, to the twelfth turn in some cases studied." In his subsequent publication² he emphasized this observation, stating that "this was a uniform process in all of the compensating tubules and it was not an atrophy but a change to a different type of epithelium." Associated with the fact that the epithelium in this region is flattened is the fact (see page 697) that "the tubule remains the normal diameter for some distance from the glomerulus," subsequently showing a marked increase in diameter. The reason for emphasizing this observation of Turley's here is that no mention of the squamous character of the epithelium in the first portion of the proximal convoluted tubule is made in the original publication by Oliver and Lund⁴ or in subsequent publications from Oliver's laboratory. However, there are indications that the latter workers have at times been dealing with the same thing in their maceration studies. For example, Oliver and Luey⁵ stated that "the tubule contiguous to the glomerulus may remain undilated, thus forming a long narrow stretch that extends to the dilated portion." Here they refer to their figure 17, which, in gross appearance at least, suggests the condition described by Turley. Also, Loomis⁶ stated that "the increase in the diameter of the proximal convoluted tubule is often less marked near the glomerulus."

While in his thesis Turley stated simply that there is "an enormous increase in the functioning capacity of one of these (hyperplastic) tubules over that of the normal tubule," in a later paper³⁷ he was more specific: "An idea of the extent of this compensatory hyperplasia can be gotten when we realize that some of these hyperplastic tubules judging by a comparison of the surface of the epithelium have the functional capacity of nine normal tubules." This is to be compared

37. Turley, L. A.: *J. Oklahoma M. A.* **14**:205, 1921.

with the statement of Oliver and Luey⁵ that "the hypertrophied unit may replace in physical size twelve normal structures."

Turley's model includes a portion of the ascending limb of Henle's loop but not the distal convoluted tubule. In figure 2 *A* it can be seen that the ascending limb has been cut off at its point of apposition to the glomerulus. Turley identified the ascending limb belonging to the nephron by its close relationship to the vas afferens of the glomerulus. From the foregoing comment, this procedure would leave no doubt as to the accuracy of the identification, particularly in view of the report by Oliver and Luey⁵ that the intimate association of the ascending limb with the glomerulus, characteristic of the normal kidney, is likewise true of the abnormal kidney.

The Illustrations.—All of the illustrations presented in this paper are new and, with the exception of figure 8, have been made in this laboratory under my direction. The legends for all figures are sufficiently explanatory, so that no further comment on them is necessary.

In Turley's original thesis there are four plates of illustrations, which will be briefly described. Plate I contains two photographs of the model of the normal nephron, a top view and a side view (the latter the same as figure 1 *A* in the present article). Plate II is a pencil drawing from the normal kidney, showing Bowman's capsule with the tubular outlet from it and cross sections of the proximal convoluted tubule, distal convoluted tubule and the ascending and descending limbs of Henle's loop. Plate III contains two photographs of the hyperplastic nephron in chronic nephritis, a top view (as in figure 2 *A* of this article) and a side view (but from the side opposite to that shown in figure 1 *B* of this article). Plate IV contains two pencil drawings labeled "hyperplastic tubules." One drawing shows Bowman's capsule with its tubular outlet, the latter exhibiting flattened epithelium, and cross sections of the proximal and distal convoluted tubules. The other drawing represents a transection of the loop of Henle, with cross sections of the ascending and descending limbs.

SUMMARY

Over twenty years ago Dr. Louis A. Turley made three plastic reconstructions of the human nephron, representing (1) the normal nephron, (2) the glomerulus and proximal convoluted tubule in acromegaly and (3) the hyperplastic nephron in chronic nephritis. These three plastic reconstructions are the subject of the present report. Turley's description of his findings is given in his own words, taken primarily from his doctoral thesis. Various aspects of his work are commented on in the light of present knowledge, and a fairly extensive series of photographs and drawings of the models is supplied. The model of the normal

nephron represents the only instance, up to the present time, in which the reconstruction of an essentially complete adult mammalian nephron, including the loop of Henle, has ever been successfully accomplished. The model of the glomerulus and proximal convoluted tubule in acromegaly is entirely unique; it demonstrates that the proximal convoluted tubule has undergone a striking increase in length, but not in diameter, and that the increase in length is roughly proportional to the enlargement of the kidney as a whole (approximately twice normal). The model of the hyperplastic nephron in chronic nephritis antedates by many years the recent model of Oliver and Lund and confirms, and to some extent supplements, their findings and the findings in maceration studies made by Oliver and Luey and by Loomis. The outstanding feature of the hyperplastic nephron is an enormous enlargement of the proximal tubule, affecting both the *pars convoluta* and the *pars recta*.

It has been known since the work of Golgi that the ascending limb of the loop of Henle applies itself to the glomerulus of the same nephron, lying in close apposition to the vascular pole. Contrary to Hamburger and Peter, who emphasized the close relationship of the ascending limb to the *vas efferens*, Turley was the first to appreciate that the essential relationship of the ascending limb is to the *vas afferens*.

ARRESTED PULMONARY COCCIDIOIDAL GRANULOMA

ALVIN J. COX, M.D.

AND

CHARLES EDWARD SMITH, M.D.

SAN FRANCISCO

For many years after the first case of coccidioidal granuloma was recognized,¹ infection by the fungus *coccidioides* (*Coccidioides immitis*) was considered to produce a progressive disease resulting uniformly in death unless the entire mass of infected tissue could be excised or at least thoroughly curetted.² However, in certain cases of coccidioidal granuloma in tissues like the leptomeninges or the joints infection must have occurred by metastasis, yet no primary focus is apparent. Four such cases of coccidioidal meningitis are included in the series of Abbott and Cutler.³ This means either that the organisms can pass into the blood stream from the site of inoculation without producing recognizable lesions or that primary lesions may be nonprogressive and become so inconspicuous that they are missed even when carefully sought at post-mortem examination. The latter possibility is supported by Abbott and Cutler's case in which the supposed primary lesion in the lung showed "remarkable degrees of healing." Giltner⁴ reported apparent healing of coccidioidal granulomas produced in calves and pigs. Dickson's⁵ collection of cases in man included an instance of "healed coccidioidal granuloma" of the lung. Further proof that coccidioidal infection may heal or become quiescent was afforded by Dickson⁶ when he established the fungus *coccidioides* as the cause of a nonfatal respiratory disease associated with erythema nodosum. This disease, known as "valley fever," "desert fever" or "desert rheumatism," is prevalent in the San Joaquin Valley, Calif., where coccidioidal granuloma has long been

From the Department of Pathology, the Department of Public Health and the Department of Preventive Medicine, Stanford University School of Medicine, and the Department of Public Health of the City and County of San Francisco.

1. Rixford, E.: *Occidental M. Times* **8**:326, 1894. Rixford, E., and Gilchrist, T. C.: *Johns Hopkins Hosp. Rep.* **1**:209, 1896.

2. Wolbach, S. B.: *Boston M. & S. J.* **172**:94, 1915.

3. Abbott, K. H., and Cutler, O. I.: *Arch. Path.* **21**:320, 1936.

4. Giltner, L. T.: *J. Agric. Research* **14**:12, 533 and 542, 1918.

5. Dickson, E. C.: *Arch. Int. Med.* **16**:1028, 1915.

6. Dickson, E. C.: *California & West. Med.* **47**:151, 1937. Dickson, E. C., and Gifford, M. A.: *Arch. Int. Med.* **62**:853, 1938.

known to occur frequently. Moreover, increasing use of the coccidioidin skin test has revealed many positive reactions in persons who live or have lived in the San Joaquin Valley but who neither give a history of erythema nodosum nor show evidence of active coccidioidal granuloma.⁷

The present study is concerned only with quiescent granulomatous lesions of coccidioidal origin. We wish to emphasize, as did Dickson, that the lesions of "valley fever" have not been demonstrated to be coccidioidal granuloma in the sense that their histologic structure is granulomatous. Careful studies are still needed to determine the relationships between various manifestations of the fungus infection.

It is to be hoped that physicians throughout the country will acquire an interest in this disease, isolated cases of which have been reported repeatedly in various parts of the world. Most of these cases have been related to residence in California, but recently indications of an endemic distribution of the fungus *coccidioides* outside California have been recorded by Woolley⁸ and by Farness.⁹ These observers have seen several patients who while living in Arizona acquired mild respiratory symptoms and erythema nodosum and whose sputum gave cultures of the fungus *coccidioides*.¹⁰ In view of the common occurrence of benign infection with the fungus *coccidioides* and the trend toward use of migratory labor in the San Joaquin Valley, as well as the extension of travel facilities within the United States and the increasing recognition of cases of this infection outside California, it seems timely to call attention to quiescent lesions due to this fungus which are likely to be passed over in postmortem examinations as tuberculous.

REPORT OF CASES

In the course of about 3,000 routine autopsies performed in San Francisco between the years 1932 and 1937 there were found 4 cases of arrested coccidioidal granuloma. In the same series there were an equal number of cases of progressive coccidioidal granuloma. It is probable that other arrested lesions were missed, since in 3 of the 4 instances which are reported here the diagnosis was first made by histologic examination of calcified nodules from the lungs or intrathoracic lymph nodes and since in many of the autopsies histologic sections from such nodules were not made as a routine. These facts indicate that cases of arrested coccidioidal granuloma in man are more frequent than is usually supposed and may considerably outnumber

7. Smith, C. E.: Unpublished data.

8. Woolley, M. T.: *J. Lab. & Clin. Med.* **23**:553, 1938.

9. Farness, O. J.: *Coccidioides* Infection: Report of a Case Primary in the Lung with Cavity Formation and Healing, *Am. Rev. Tuberc.*, to be published.

10. Farness, O. J.: Personal communication to the authors. Woolley, M. T.: Personal communication to the authors.

the cases of fatal progressive granuloma. It should be noted particularly that the autopsies were performed in a district outside the San Joaquin Valley. There, where active lesions of this disease are frequent, the incidence of arrested lesions may be expected to be much higher than is indicated by their frequency in our autopsy series.

In all cases to be reported the patients were past 50 years of age. In 2 instances the occupation was such that there might have been unusual exposure to dust, but in only a single instance (case 4) was the patient known to have spent a period of time in the San Joaquin Valley. In no patient were the arrested coccidioidal lesions a factor in causing death.

CASE 1.—A 56 year old white miner had worked in dusty coal mines, as well as in gold and silver mines, for thirty-five years up to seven years before his death. Residence in the San Joaquin Valley was not recorded. He denied having had any severe illnesses. Roentgen studies of the chest three years before death showed a dense shadow about 2 cm. in diameter at the apex of the right lung and an indefinite faint shadow at the apex of the left lung.

At autopsy, performed the day after death, the anatomic diagnosis was: carcinoma of the lung with metastases to the tracheobronchial lymph nodes; bronchopneumonia; emphysema; arrested coccidioidal granuloma of the lungs.

A large infiltrating tumor almost replaced the lower lobe of the right lung, and at the bronchial bifurcation the lymph nodes were enlarged and grossly infiltrated with tumor. The posterior parts of both lungs showed extensive hyperemia, edema and pneumonia. In addition, there was a firm black nodule, about 2 cm. in diameter, in the lung substance near the apex of each lung. Each nodule contained a core of gray material, 1.4 cm. in diameter. There were no other scars.

Histologically, each of the two nodules from the upper lobes was composed largely of masses of partly calcified granular material, surrounded by a well demarcated dense fibrous capsule. The inner portion of this capsule was extensively hyalinized; the outer portion contained much black carbon pigment, scattered lymphocytes and several small nodules. The last mentioned varied in composition from hyalinized fibrous tissue to fibrous tubercles, in which epithelioid cells and occasional giant cells of the Langhans type were recognizable. However, there was no evidence of active extension of the process (fig. 1). Toward the periphery of the caseous material and embedded in the fibrous tissue at several points were a few scattered spherules from 15 to 40 microns in diameter. Each of these had a granular inner structure and a double-contoured refractile capsule characteristic of the fungus *coccidioides*. At the center of the caseous material were a number of empty refractile capsules like those of the intact spherules near the periphery. Calcified spherules were not seen, but some of the spherules present showed irregular thick capsules, the outer portions of which were nonrefractile and were stainable with eosin, giving the surface a ragged appearance. One spherule contained endospores (fig. 2). The hilar and mediastinal lymph nodes contained tumor tissue, and one node showed several small scars, but there was no evidence of coccidioidal infection.

Comment.—The character of the pulmonary nodules was not suspected in examination of the gross specimen, and no cultures were made. Of particular interest was the presence of a localized lesion in *each* lung with no evidence of

lymphatic spread. These lesions were comparable in size and position to the shadows seen in the roentgen films taken three years before death. At that time they were presumably already old, and apparently they underwent no significant change during the last three years of the patient's life. However, the presence of intact spherules in the lesions suggests that some organisms were still alive.

CASE 2.—This 66 year old Scotch deliveryman was seen over a period of two years before death, but an inadequate history was recorded, and it is not known whether he had lived in the San Joaquin Valley. Roentgen examination of the chest two years before death showed no abnormality of the lung fields. The

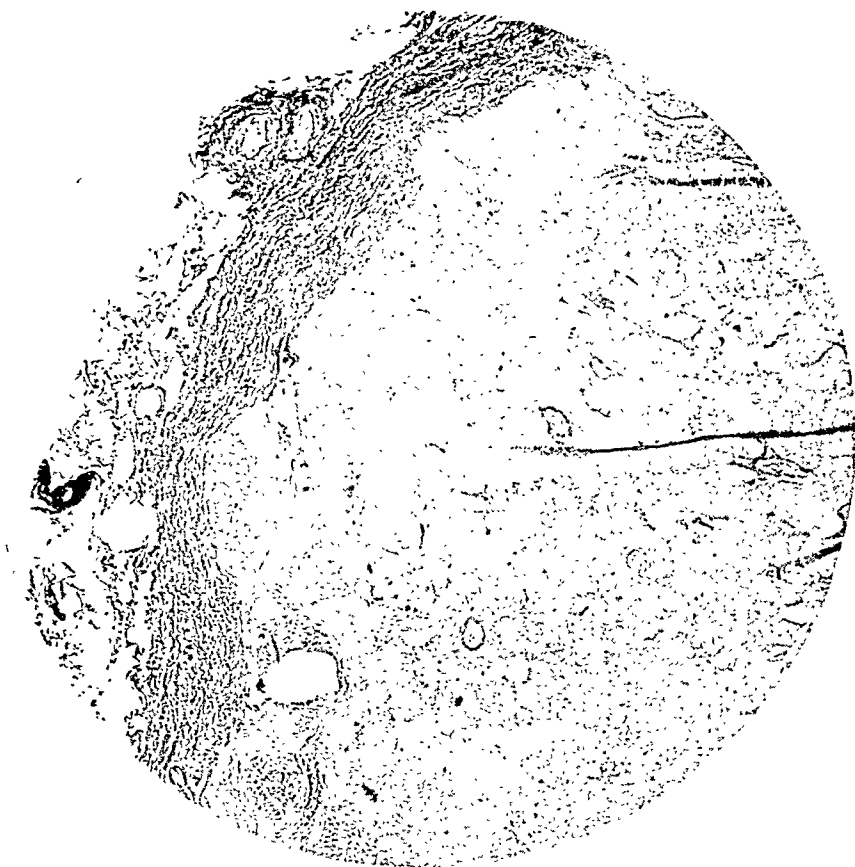


Fig. 1.—Low power magnification of the margin of one of the pulmonary nodules in case 1, showing the dense fibrous capsule and a small secondary nodule adjacent to a small artery at the outer surface. Hematoxylin-Van Gieson stain; $\times 13$.

patient died following a colostomy performed to relieve a chronic obstruction of the large bowel.

Autopsy, performed eight hours after death, showed: diffuse hypertrophy of the colon without obstruction; bronchopneumonia; arteriosclerosis of the coronary arteries; arrested coccidioidal granuloma of the lung.

There was moderate bronchopneumonia in both lungs posteriorly, and at each apex was a moderate-sized dense fibrous scar. One was composed of an oval caseous central portion, 8 mm. long, and a capsule of dense black fibrous tissue, about 1 mm. thick.

Histologic examination showed many clusters of black pigment in the outer portion of the fibrous capsule, which was hyalinized and sharply demarcated from the adjacent lung tissue. It contained scattered clusters of lymphoid cells but no epithelioid or giant cells. The centrally located caseous material was granular and basophilic. It contained numerous long clefts resembling spaces from which crystals had been dissolved, and many easily recognizable spherules, from 15 to 30 microns in diameter, with double-contoured refractile capsules characteristic of the fungus *coccidioides*. Intimately attached to the outer surface of some spherules was an irregular thin layer of homogeneous, slightly basophilic material, which gave



Fig. 2.—A portion of the caseous material from the nodule in case 1, showing a thin-walled spherule containing endospores and a smaller one with a thickened irregular capsule. Hematoxylin-eosin stain; $\times 490$.

the surface a rough, ragged appearance. Near the periphery of the caseous zone were a few completely calcified spherules, stained deeply with hematoxylin. Two spherules were larger than the others, had thin capsules and contained numerous small round endospores characteristic of the fungus *coccidioides*.

Comment.—Since the unusual nature of the nodules in the lungs was not recognized during life or at autopsy, no cultures were made, no material was taken for histologic study from the fibrous area in the other lung, and no special search was made for abnormal lymph nodes within the thorax. However, none

was found on routine examination. Although the lesion described was completely arrested, one could not consider it healed, since numerous intact spherules were present. Presumably the calcified spherules were dead before the death of the patient, but many spherules deviated in appearance from those in fresh lesions only by the accumulation of an irregular thin homogeneous stainable layer on the surface of the capsule.

CASE 3.—A Greek insurance broker aged 53 years gave no history of residence in the San Joaquin Valley and remembered no serious illness prior to the onset of heart failure seventeen months before death. Roentgen examination of the chest was not made.

Autopsy, performed four hours after death, gave the following anatomic diagnosis: generalized arteriosclerosis, with hypertrophy of the heart and scars in the heart muscle; chronic passive hyperemia of the viscera; anasarca; chronic cholecystitis with calculi in the gallbladder; scar at the apex of the lung; arrested coccidioidal granuloma of a mediastinal lymph node.

The lungs showed no lesions aside from peripheral collapse and hyperemia, with a small superficial scar at the apex of the right lung, where there were a few fibrous pleural adhesions. The hilar lymph nodes were small and black, except for one which measured 2 by 1 by 1 cm., behind and to the right of the trachea, near its bifurcation. This node contained an irregular white caseous mass, about 7 mm. in greatest diameter, with a peripheral firm calcified portion and a thin but dense fibrous capsule.

Histologic study of this lesion showed a granular structure of the caseous material, in which were many slender fusiform clefts. Here and in many places within the inner portion of the fibrous capsule were many spherules with double-contoured refractile capsules, from 10 to 30 microns in diameter. In some the outer surface of the capsule was slightly ragged and irregular. Many capsules were empty, but others contained poorly defined refractile granular material, and some were calcified, showing a deep blue staining reaction with hematoxylin and lacking visible detail in their inner structure. No small endospores were found, but in two places spherules buried in the capsule enclosed daughter spherules up to 10 microns in diameter. The fibrous capsule was extensively hyalinized in its inner portion. In one place it had a nodular appearance, as if formed by fusion of several masses, but no cellular tubercles were present. Aside from a few scattered lymphocytes, the capsule contained few cells. It was sharply demarcated from the remaining lymphoid tissue.

Comment.—Presumably the portal of entry of the fungus was the lung, though no lesion was found except the apical scar, which was considered so characteristic of a tuberculous scar that no material was saved for histologic study. It did not grossly resemble any of the arrested lesions found in the other cases reported here. Conceivably, the pulmonary lesion was completely healed or had been reduced to such insignificant proportions that it was overlooked. Also, the possibility cannot be ruled out that the organism passed through the lung without producing a lesion. In any event, the lesion of the lymph node was completely arrested, as determined by its histologic structure, but, as in cases 1 and 2, the lesions cannot be considered healed.

CASE 4.—A 60 year old Yugoslavian farm hand came to California in 1917 and was employed on a hopper machine in the San Joaquin Valley. Soon he noticed on the dorsum of his right wrist an eczematous lesion. This became ulcerated and persisted until his death, fifteen years later, though during the

intervening years it had undergone stages of partial healing and exacerbation. On several occasions during the early course of this lesion cultures from it showed growth of *Coccidioides immitis*. The patient had no pulmonary symptoms except those associated with heart failure during the last two years of his life. However, a roentgenogram of his chest made two years before death showed several "round calcified areas of density the size of marbles" in both lower lung fields, with moderate increase in the hilar markings (fig. 3).

Autopsy was performed two days after death. The anatomic diagnosis was: generalized arteriosclerosis with hypertrophy of the heart and scars in the heart muscle; thrombosis of the renal artery with infarction of the kidney; arrested tuberculosis of a mesenteric lymph node; scars at the apexes of both lungs; arrested coccidioidal granuloma of the skin of the wrist and of the lungs.

The lesion of the wrist consisted of an irregular zone of thin, slightly uneven skin, pigmented light brown, about 3 cm. in diameter, the center of which was covered by a small crust. No pus was present, and no material was removed for histologic study. There were a few firm localized adhesions partly obliterated

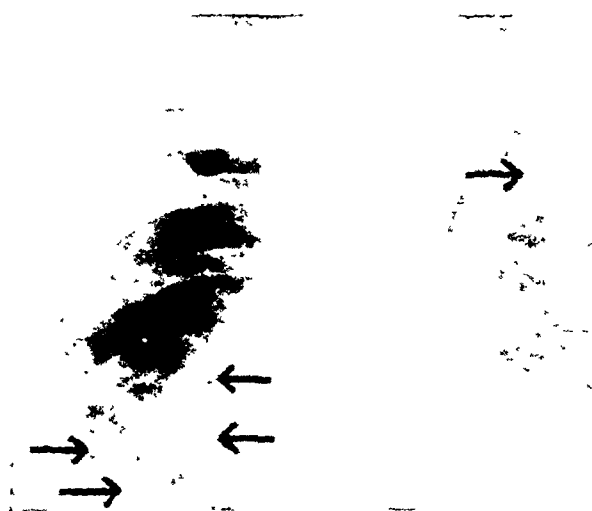


Fig. 3.—Roentgenogram of the chest (case 4) taken two years before death. There are well circumscribed areas of density in the lower portions of both lung fields.

ing the left pleural cavity posteriorly and covering a calcified subpleural nodule, about 1 cm. in diameter, near the upper margin of the lower lobe of the left lung posteriorly. There was a similar large node, measuring 3 by 2.5 by 2.5 cm., in the tissue of the same lower lobe anteriorly near the inferior margin, and two smaller, pea-sized nodules were found in the periphery of the upper lobe near its lower lateral margin. The right lung was bound to the parietal pleura anteriorly and inferiorly by fibrous adhesions and showed an easily visible scar at the apex. There were interlobar adhesions, and near the periphery of the middle lobe anteriorly was a nodule, 1 cm. in diameter, like those on the left. Two similar nodules, 1.5 cm. in diameter, were present anteriorly and posteriorly in the lower portion of the lower lobe. All of the nodules had sharply defined dense fibrous capsules, 1 to 2 mm. thick, and a central portion composed of dry white opaque grumous material, partially calcified (fig. 4). They were similar to the lesions described in the previous cases. There was no enlargement of the hilar lymph nodes. The mesentery contained a calcified mass, 1.5 by 0.8 by 0.8 cm., which

was found on routine examination. Although the lesion described was completely arrested, one could not consider it healed, since numerous intact spherules were present. Presumably the calcified spherules were dead before the death of the patient, but many spherules deviated in appearance from those in fresh lesions only by the accumulation of an irregular thin homogeneous stainable layer on the surface of the capsule.

CASE 3.—A Greek insurance broker aged 53 years gave no history of residence in the San Joaquin Valley and remembered no serious illness prior to the onset of heart failure seventeen months before death. Roentgen examination of the chest was not made.

Autopsy, performed four hours after death, gave the following anatomic diagnosis: generalized arteriosclerosis, with hypertrophy of the heart and scars in the heart muscle; chronic passive hyperemia of the viscera; anasarca; chronic cholecystitis with calculi in the gallbladder; scar at the apex of the lung; arrested coccidioidal granuloma of a mediastinal lymph node.

The lungs showed no lesions aside from peripheral collapse and hyperemia, with a small superficial scar at the apex of the right lung, where there were a few fibrous pleural adhesions. The hilar lymph nodes were small and black, except for one which measured 2 by 1 by 1 cm., behind and to the right of the trachea, near its bifurcation. This node contained an irregular white caseous mass, about 7 mm. in greatest diameter, with a peripheral firm calcified portion and a thin but dense fibrous capsule.

Histologic study of this lesion showed a granular structure of the caseous material, in which were many slender fusiform clefts. Here and in many places within the inner portion of the fibrous capsule were many spherules with double-contoured refractile capsules, from 10 to 30 microns in diameter. In some the outer surface of the capsule was slightly ragged and irregular. Many capsules were empty, but others contained poorly defined refractile granular material, and some were calcified, showing a deep blue staining reaction with hematoxylin and lacking visible detail in their inner structure. No small endospores were found, but in two places spherules buried in the capsule enclosed daughter spherules up to 10 microns in diameter. The fibrous capsule was extensively hyalinized in its inner portion. In one place it had a nodular appearance, as if formed by fusion of several masses, but no cellular tubercles were present. Aside from a few scattered lymphocytes, the capsule contained few cells. It was sharply demarcated from the remaining lymphoid tissue.

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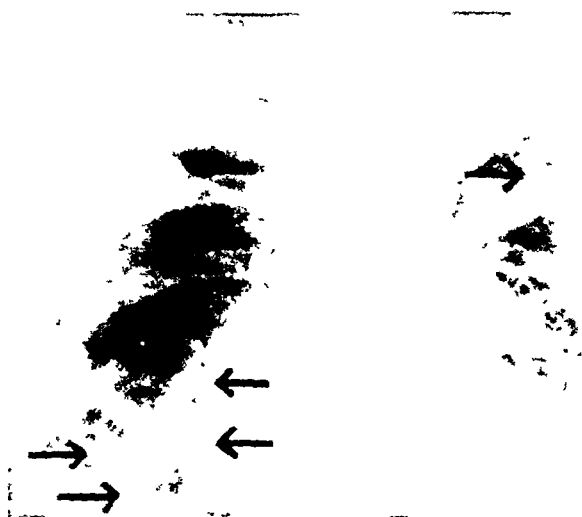


Fig. 3.—Roentgenogram of the chest (case 4) taken two years before death. There are well circumscribed areas of density in the lower portions of both lung fields.

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on histologic examination showed extensive caseation of the central portion and beginning ossification at the periphery, but no demonstrable bacteria or fungi.

Histologically the lesions in the lungs were sharply separated from the adjacent normal-appearing tissue by a dense fibrous tissue wall; the inner portion was extensively hyalinized. One of the lesions showed remnants of several fused smaller nodules. The central portions were made up of granular and amorphous material, stained extensively with hematoxylin and containing many slender clefts. There were a few free spherules with double-contoured refractile capsules, from 15 to 30 microns in diameter. Some were calcified and stained deep blue with hematoxylin, some were empty, and some contained poorly defined granular material. Several contained typical endospores of the fungus *Coccidioides*. Cultures of material from the pulmonary lesions on Sabouraud's dextrose agar showed growth of mycelia identified as *Coccidioides immitis*. The fibrous capsule was infiltrated by a few lymphoid cells, but there were no tubercles or other evidence of active extension of the fungus infection. Occasional spherules were embedded in the hyaline portion of the fibrous capsule, but there was no local inflammatory reaction, and such organisms showed no endosporulation. Several peribronchial

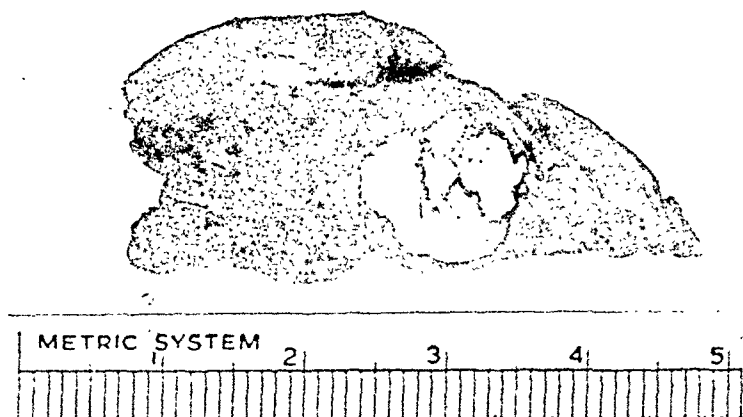


Fig. 4.—Section through a partially calcified caseous arrested coccidioidal granuloma of the lung (case 4).

lymph nodes were slightly enlarged, but no coccidioidal lesions were found. The calcified lesion in the mesentery, though structurally resembling the nodules in the lungs, contained no demonstrable organisms, so it was considered as probably the result of a primary tuberculous infection.

Comment.—Death was apparently due to circulatory failure and certainly was unrelated to the lesions produced by the fungus *Coccidioides*. The relation between the lesion of the skin and those in the lungs cannot be decided with certainty. It is tempting to consider the cutaneous lesion as a primary focus from which organisms reached the lung by the blood stream or by lymphatic channels, but there was no history of an injury to the skin preceding the development of the lesion, and no evidence was found at any time to indicate disease of the axillary lymph nodes. Furthermore, similar lesions of the lungs were found in the preceding cases without any associated cutaneous lesions. We believe that secondary infection of the skin from a primary lesion of the lung is a likely possibility, though it is possible that a primary lesion of the skin had always remained localized, and the pulmonary process resulted from a separate infection of the respiratory tract. Regardless of the sequence of events in this case, it is prob-

able that the lesions in the lungs represented an infection of many years' duration, perhaps as long as fifteen years, the known duration of the cutaneous lesion. It is especially notable that cultures showed living organisms in this patient who had carried localized coccidioid lesions for fifteen years.

EXPERIMENTAL OBSERVATIONS

In view of the obviously arrested nature of the lesions described, experiments were planned with a view to producing arrested lesions in animals. It was not our purpose to reproduce the human disease in all details or to evaluate the influence of immunity and allergy in the animals, though further studies are in progress in this direction.

Almost all experimental work heretofore reported in the literature, as well as that previously carried out in this institution, has been done by inoculating animals with quantities of material which were not accurately standardized, such as suspensions of material obtained from cultures. The lack of readily measurable units because of the presence of both mycelial filaments and chlamydo spores makes accurate calibration of the inoculation difficult. Material obtained from pus from animal lesions offers a simpler means of estimation and calibration of the amount of infectious material to be inoculated. Here, owing to the occurrence of the organisms as spherules, it is possible to count directly the units in a given sample of pus and thus calculate the approximate number to be injected into a test animal.

In our experiments material for inoculation was obtained from testicular lesions in guinea pigs, each of which had been infected intratesticularly with 0.25 cc. of a saline suspension of a virulent culture of the fungus coccidioides (S. F. S. no. 46) grown on Sabouraud's medium. To estimate the concentration of spherules, a sample of diluted pus was filtered through sterile cotton and mixed with an equal volume of 10 per cent sodium hydroxide solution to dissolve debris, and the spherules in this cleared solution were counted in an ordinary blood counting chamber. Recently we have found that partial separation of the spherules from the other elements of pus can be obtained by centrifugating the diluted pus. The spherules accumulate at the bottom of the sediment in the centrifuge tube.

There are, of course, errors involved in such calibration of a suspension of spherules. Some spherules may be dead, and variations in the number of free endospores cannot be detected by counting spherules. However, our experience has shown such factors to be reasonably constant in different samples of the same pus and in pus from different animals, so that estimations of the number of growing units are all of the same order of magnitude.

Injection of the organisms into the venous circulation of young adult white rats was preferred to administration by surface application or inhalation, as it was felt that the number of spherules reaching the

tissues could be most accurately estimated in this way. Although the analogy to the arrested lesions in the cases observed in man is not exact, the lungs were predominantly affected in both man and animal, and in the experiments an advantage was gained by knowing that all injected organisms reached the tissues. The injections were made into the right side of the heart or into the jugular vein. Most of the animals were anesthetized for the injection with ether, though others were anesthetized by intraperitoneal injection of pentobarbital sodium. No difference in the development of lesions could be detected in these two groups of animals, so it has been assumed that a short exposure of the tissues to ether during narcosis does not affect the development of the fungus *coccidioides* within them. Further work has shown that rats differ very little in susceptibility from guinea pigs, which are well known to be highly susceptible to infection with this fungus.

Effect of Intravenous Inoculation of Spherules of Coccidioides

Spherules Injected	Animals	Average Duration of Life, Days	Loss of Weight
30,000 or more.....	6	9	Rapid
20,000.....	4	13	Rapid
10,000.....	2	48	Slow, continuous
5,000.....	5	*	Slight, transient
2,500.....	4	*	Slight, transient
1,000.....	5	*	Slight, transient
750 or less.....	20	*	Slight, transient

* After a few days the animals appeared normal and gained weight steadily. They died of causes unrelated to the granulomatous lesions, which were arrested. One exception is discussed in the text.

To determine approximately the susceptibility of the rats, calibrated suspensions of organisms were injected into 43 animals in the manner described. The inoculums ranged from 100 to 150,000 spherules. As is apparent from the accompanying table, the minimum fatal inoculum was 10,000 spherules. The lungs of animals receiving fatal inoculums uniformly showed extensive coccidioidal granuloma. One animal which had received 1,000 spherules lost weight and died three weeks after infection. Its lungs showed widespread coccidioidal lesions. This was the only animal of those receiving 5,000 spherules or less whose death could be attributed to coccidioidal granuloma. Three animals which had received small inoculums died within two months of secondary infections, but in these the coccidioidal lesions were not extensive. With exclusion of these 4 animals, 21 rats which received 1,000 spherules or less survived periods averaging four hundred and thirty-nine days per rat, with no significant difference related to the dose. One rat became pregnant and produced a normal litter without showing any evidence of disease.

Granulomatous lesions containing spherules of the fungus *coccidioides* were found in all of the 21 animals but 2. One of these, which had received 500 spherules and died after two hundred and forty days, was not examined post mortem. The other showed no granulomatous lesions on careful gross and histologic study of the organs three months after injection of 100 spherules. The lungs of the other 19 animals all showed irregular yellowish to white opaque nodules from 1 to 3 mm. in diameter (fig. 4). These were most numerous at the pleural surface and were readily visible before the lungs were sectioned, but, in spite of this relation to the pleura, in only a few instances were there any pleural adhesions. The mediastinal lymph nodes were enlarged in most animals, and a few contained grossly visible, poorly defined small white opaque nodules.

Accurate counts of the number of lesions found in the lungs of the different animals were not made, since some of the lesions were confluent and there was sometimes difficulty in distinguishing grossly between specific granulomatous nodules and small scars or abscesses in the lungs due to other causes. It was noted, however, that there was a correlation between the number of spherules injected and the number of lesions produced, though the number of lesions was always smaller than the estimated number of spherules. In some instances not more than 10 per cent of the estimated spherules were represented by nodules in the lungs.

In order to study the evolution of the fibrous nodules without introduction of differences in size of the inoculum as a confusing factor, a second group of 33 rats was inoculated in the manner already described. Each animal received 200 spherules by right-sided intracardiac injection. Twelve animals were put to death during the first four months. Eighteen were examined post mortem at approximately monthly intervals during the succeeding fourteen months. Three were studied at nineteen, twenty-two and twenty-nine months, respectively. Eight of the animals died of secondary infections during the period of observation, but, although the appearance of the lungs was frequently altered by pneumonia, the structure of the granulomatous lesions was not appreciably different from that in the animals which were put to death.

Two animals, which lived for one and seven months, respectively, after inoculation, showed no gross or microscopic lesions in the lungs, mediastinal lymph nodes, spleen, kidneys, liver, heart or testis. The reason for the failure of the organisms to proliferate is not known. Thirty-one of the animals showed lesions in the lungs grossly like those described in the previous series, though there was quite striking variation in the number of gross lesions in different rats. This was unrelated to the time following inoculation. Presumably it was a manifestation of varying degrees of resistance in different animals.

Nature of the Lesions.—Within two weeks after inoculation, nodular lesions from 1 to 2 mm. in diameter had developed in the lungs, due to the accumulation of masses of epithelioid cells, among which were a few giant cells of the Langhans type. These nodules were irregular in contour, owing to conglomeration of smaller cellular tubercles. In the larger lesions caseous necrosis of the central part had occurred, and the necrotic areas contained disintegrating polymorphonuclear leukocytes and nuclear débris. A broad zone of lymphocyte and macrophage infiltration containing scattered eosinophilic leukocytes surrounded the epithelioid cell masses at this stage. Spherules of the fungus coccidioides in all stages of development were abundant not only in the central

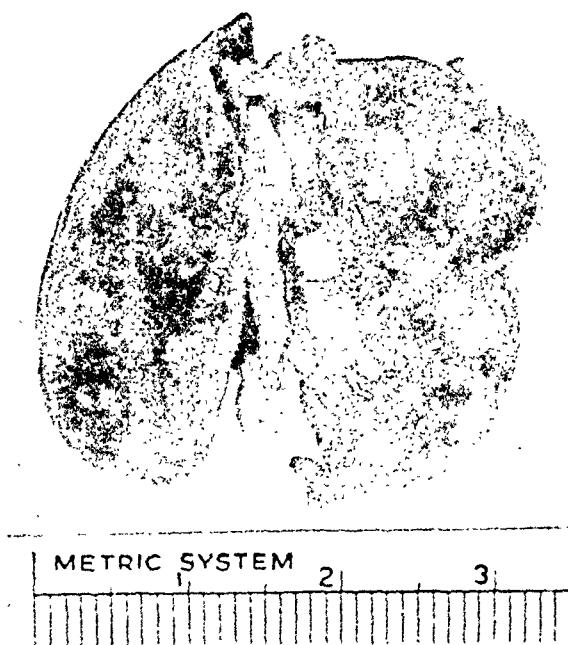


Fig. 5.—Lungs of an adult white rat fourteen months after intravenous inoculation of 1,000 spherules of the fungus coccidioides.

portions of these lesions but also among the epithelioid cells throughout all portions of the tubercles and within giant cells.

In animals killed a longer time after inoculation the conglomerate nature of the nodules was more marked, suggesting that spread of the lesions had continued after the first two weeks. At two months significant amounts of collagen could be seen among the epithelioid cells in the tubercles, which had become distinctly less cellular. Abundant lymphoid cells were still grouped peripherally about the nodules, and spherules of the fungus coccidioides were scattered throughout all parts of the tubercles (fig. 5).

A gradual increase in the amount of collagen with an accompanying decrease in cellularity and a decrease in the degree of peripheral lymphocytic infiltration occurred during the remainder of the twenty-nine month period of observation. Calcification of the caseous material was observed in about one third of the animals killed five months or more after inoculation. In the lesions nine months old definite localization of the spherules to the centers of the tubercles was noted. This became more pronounced as the lesions became older until, after two years, most of the spherules were deeply embedded in the fibrous tissue of the



Fig. 6.—Experimental coccidioidal granuloma two months old in the lung of a white rat. Hematoxylin-eosin stain; $\times 57$.

nodules (fig. 6). This gave the impression that the organisms were mechanically limited in their growth by the dense fibrous tissue of the nodules. Another interesting appearance, presumably due to mechanical limitation of the growth of the organisms, was that of three generations of spherules growing one within another (fig. 7). This was seen frequently in the older experimental lesions, though it was not certainly identified in the human lesions. Figure 7 also shows striking irregularity in the size of developing sister spherules from endospores within a single old shell, another common observation in the old lesions. A third char-

acteristic of the spherules in the old lesions, and one which was also present in the human cases, was a thickening of the capsule, the outer surface of which frequently had an irregular, ragged appearance due to a layer of slightly eosinophilic material unlike the refractile substance forming the inner portion of the capsule.

The oldest lesions were distinctly larger than those a few months old, and they showed the most extensive conglomeration of small noncaseous tubercles, as well as the least caseation. In many the peripheral tubercles in a conglomerate nodule were obviously more cellular than those more

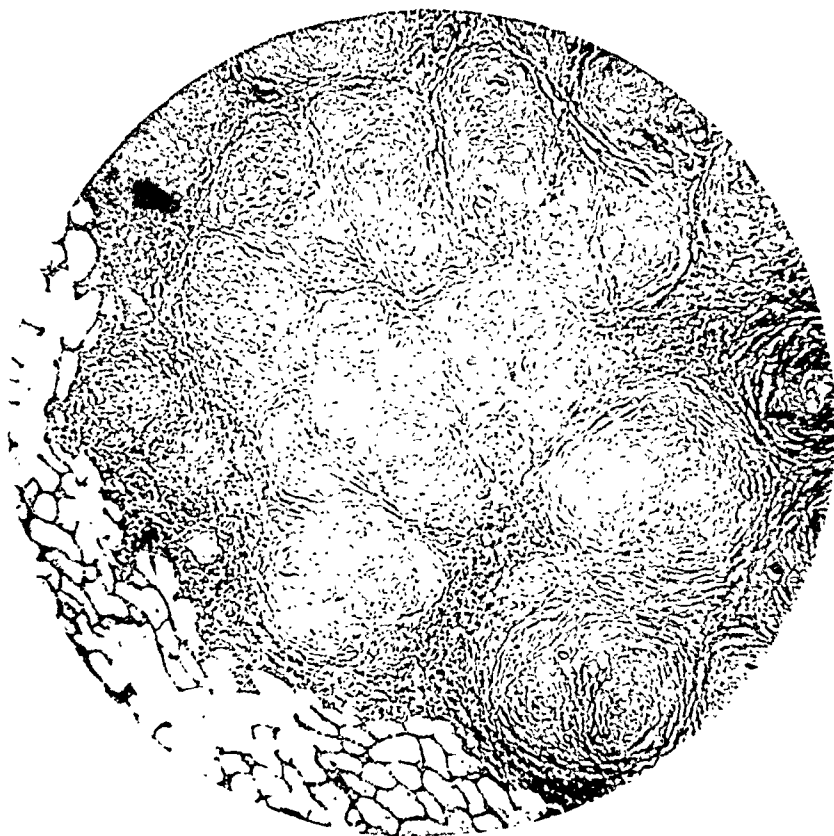


Fig. 7.—Experimental coccidioidal granuloma twenty-two months old in the lung of a rat. The lesion is composed largely of hyalinized fibrous tissue and shows little evidence of inflammation, but spherules of the fungus are abundant. Hematoxylin-eosin; $\times 57$.

centrally placed, and it was noted occasionally even in the oldest lesions that a few spherules existed in tissue completely outside the fibrous nodules. Such spherules were enclosed by giant cells. This indicates that even though the infection is controlled, a limited spread of the organisms may occur. Since the lesions in animals dying of pneumonia or some other complication were not appreciably different from those in animals which were put to death, such spread cannot be considered a flare-up during a secondary infection.

In 29 of the 31 animals with pulmonary coccidioidal granuloma the mediastinal lymph nodes showed lesions. These lesions were similar to those in the corresponding lungs, indicating that they developed coincidentally with the lesions in the lungs. The structure of some of the lesions in the lymph nodes likewise suggested a slow local spread. Cultures were made from the granulomatous lesions in 12 animals of the first series and 23 of the second series by grinding one or more excised nodules in a mortar and planting the material obtained on

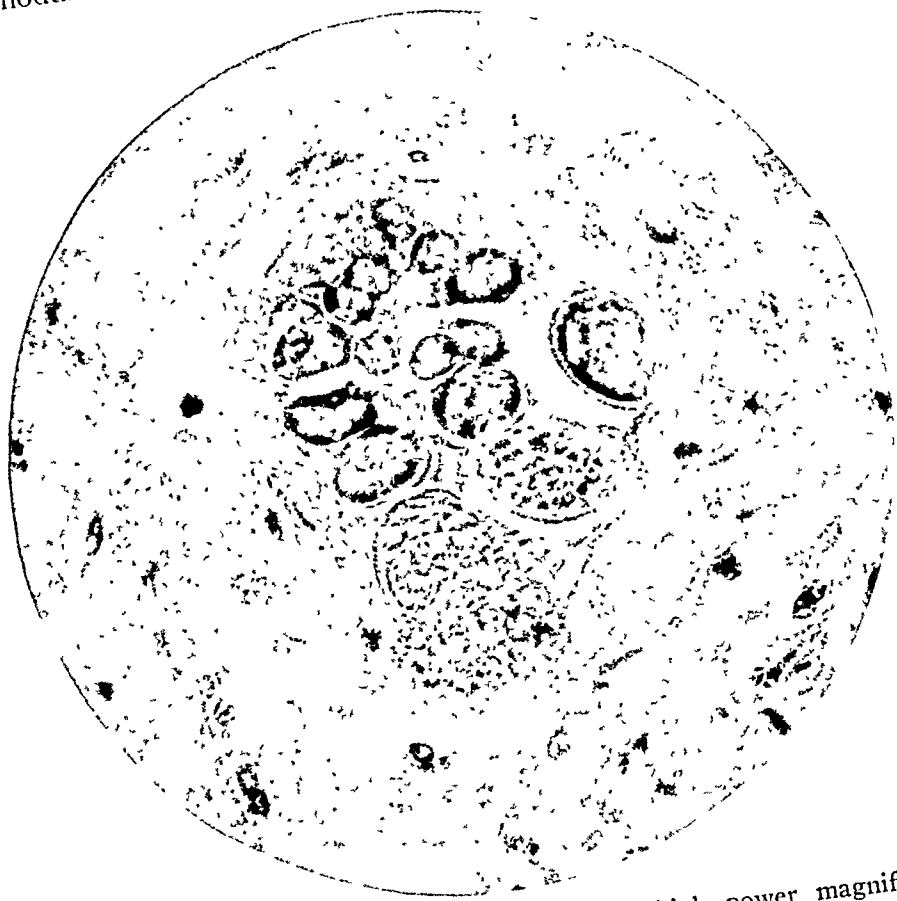


Fig. 8.—Experimental coccidioidal granuloma (high power magnification of the lesion shown in figure 7). Three generations of spherules are seen limited to one region. At the edge of the group of well developed spherules are refractile remnants of an old capsule. The endospores in two of the spherules represent the third generation.

Sabouraud's dextrose agar. Sixteen of the cultures were from animals which had lived longer than twelve months following inoculation. One had survived two and a half years. In 31 instances there was growth of the fungus *coccidioides* from the lesions in the lungs; in 2, growth occurred from nodules from lymph nodes but not from those from the lung, while in 2 others cultures produced no growth of fungus.

One of these concerned an animal which died with extensive bronchopneumonia seven months after inoculation of 200 spherules. Histologic study of the lungs from this animal showed that the lesion which had been cultured was a pyogenic abscess with a fibrous wall, while lesions due to the fungus were quite small and may not have been included in the material cultured. The second negative culture was obtained from an animal which had been killed two months after inoculation. No explanation can be given for the failure of the organisms to grow. It can be seen that with two possible exceptions all of the animals harbored viable fungi and that living organisms remained in the lesions for as long as two and a half years. This again emphasizes the fact that although the lesions were arrested, they cannot be considered to have healed.

GENERAL COMMENT

In the 4 cases of arrested coccidioidal granuloma in man which are reported in this paper there was no characteristic position of the lesions in the lungs. They occurred as frequently in the upper as in the lower lobes. Each was near the periphery of the lung, but all did not lie immediately beneath the pleura. Definite involvement of lymph nodes was demonstrable only in case 3, in which the only lesion found was in a large lymph node at the hilus of the lung. It is possible, however, that one or more of the nodules in case 4 represented completely destroyed lymph nodes in the lung substance. The lesions uniformly consisted of relatively large central masses of partially calcified caseous material with surrounding sharply outlined capsules of dense hyalinized connective tissue. In 2 cases small fibrous tubercles were present in the capsules, and most of the lesions were lobulated, suggesting that they had been formed as conglomerate nodules. It is possible that smaller lesions with less prominent central caseous areas may result from arrest or healing of coccidioidal granuloma. Such lesions may be easily missed at autopsy, since they cannot be distinguished grossly from arrested or healed lesions of tuberculosis.

Specific organisms were cultured in only a single case of the series in man, but in the others the presence of numerous characteristic spherules in histologic sections permits only the diagnosis of coccidioidal granuloma. Although some of the spherules were calcified and others showed no internal structure, in every lesion there were spherules resembling those seen in actively progressive coccidioidal granuloma, and in each case a few spherules contained endospores. This strongly suggests that viable organisms were present, as was proved in case 4 by culture and by animal inoculation. Whether spread of the fungus occurs from such arrested lesions cannot be decided from our human material, but evidence that the organisms readily enter lymphatic vessels

is afforded by our experimentally inoculated animals, more than 90 per cent of which showed secondary infection of mediastinal lymph nodes. It is probable that caseous lesions are sometimes sources of massive infection of the blood stream, as has been demonstrated in cases of tuberculosis. It is our opinion that the arrested lesions, whether in the lungs or in the lymph nodes, constitute potential sources of widespread infection.

The influence of allergy and immunity in the formation of arrested lesions cannot be deduced from the appearance of the lesions in our cases, nor can it be stated with certainty whether these lesions represented primary or secondary infections. In a number of respects they resembled the reaction of the tissues to a primary tuberculous infection: The pulmonary lesions were localized; they were situated peripherally in the lungs, without constant relationship to the apexes; caseation of the lesions in lymph nodes was prominent in 1 case, and all lesions showed extensive central caseation with calcification. The hematogenous route of infection of the lungs cannot be assumed for most cases in man, though the history of case 4 suggests that the infection of the lung may have been hematogenous.

The arrested lesions produced by intravenous inoculation of rats with small numbers of spherules of the fungus *coccidioides* were comparable to the lesions found in man so far as they were localized pulmonary lesions which contained viable fungi and were associated with similar lesions in the regional lymph nodes. The formation of conglomerate nodules was much more prominent in the lungs of the rats, whereas caseation in old lesions was less extensive in the rats than in man. However, the experimental lesions of long duration had striking basic similarities to the lesions in human lungs.

Although no evidence was obtained that the number of spherules in a minimum fatal inoculum differed widely in different animals, there was a definite indication of difference in response of individual rats to a small inoculum of 200 spherules. A similar individual variation in response to infection in man may reasonably be expected.

SUMMARY

Four cases of completely arrested coccidioidal granuloma of the lungs or of the bronchial lymph nodes in man are reported. In none of these were the granulomatous lesions a factor in the cause of death.

Similar arrested lesions could be produced at will by injecting small numbers of spherules of a virulent strain of the fungus *coccidioides* intravenously into white rats or guinea pigs. The minimum fatal inoculum for rats inoculated in this way was approximately 10,000 spherules.

In nearly all the arrested experimental lesions organisms remained viable for periods up to two and a half years. In a human case organisms were cultured fifteen years after they had been first demonstrated.

It is concluded that instances of arrested coccidioidal granuloma are more frequent than has heretofore been realized, that they probably outnumber the fatal cases of this disease and that they are not restricted to the region of the San Joaquin Valley, Calif. The relationship of arrested coccidioidal granuloma to the benign clinical disease known as "valley fever" is as yet unknown. Although the lesions present a histologic picture of inactivity, they may contain viable fungi for many years and constitute a possible source of dissemination.

Case Reports

DIVERTICULA OF THE VERMIFORM APPENDIX ASSOCIATED WITH AN OVERGROWTH OF NERVE TISSUE AND A PARTIAL MUCOCELE

JACOB R. DORDICK, M.D., NEW YORK

One finds very few cases of diverticula¹ of the vermiform appendix recorded in the literature up to 1907.² This is not astonishing because at or about this time the clinical and pathologic concepts of appendicitis were first being molded.³ Since then, several excellent reviews have been presented.⁴ The authors of these reviews, utilizing for the most part surgical material, collected 86 instances. To date, a total of 126 cases have been recorded.⁵ This probably does not represent all the cases, since many may not have been reported.

The case reported now warrants presentation because of the large number of closely placed diverticula, the unusual overgrowth of nerve tissue and the partial mucocele. I have been unable to find any record of a similar one in the literature.

From the Department of Pathology, Beth Israel Hospital.

1. The term "diverticula" is used here for convenience sake, without regard to completeness or incompleteness of the layers forming the wall.

2. Isabelle Herb was able at that time to collect 25 instances from the literature (Tr. Chicago Path. Soc. 7:94, 1907).

3. Kelly, H. A., and Hurdon, E.: *The Vermiform Appendix and Its Diseases*, Philadelphia, W. B. Saunders & Company, 1905. Murphy, J. B.: *J. A. M. A.* 22:302, 1894.

4. (a) McCarty, W. C., and McGrath, B. F.: *Surg., Gynec. & Obst.* 12: 211, 1911. (b) Moschcowitz, E.: *Ann. Surg.* 63:697, 1916. (c) Stout, A. P.: *Arch Surg.* 6:793, 1923. (d) Mulsow, F. W.: *ibid.* 24:923, 1932. (e) Edwards, H. C.: *Brit. J. Surg.* 22:88, 1934. (f) Sauer, P. K.: *Am. J. Surg.* 10:564, 1930. (g) Collins, D. C.: *Ann. Surg.* 104:1001, 1936.

5. (a) Seelig, M. G.: *Ann. Surg.* 44:78, 1906. (b) Schweizer, R.: *Virchows Arch. f. path. Anat.* 185:278, 1906. (c) Konjetzny, G. E.: *München. med. Wchnschr.* 56:2251, 1909. (d) Simon, W. V.: *Berl. klin. Wchnschr.* 48: 1501, 1911. (e) Krabbel, M.: *Beitr. z. klin. Chir.* 80:121, 1912. (f) Wilkie, O. P. D.: *Brit. J. Surg.* 8:392, 1921. (g) Löhr, W.: *Deutsche Ztschr. f. Chir.* 171:30, 1922. (h) Chase, W. H.: *Canad. M. A. J.* 17:416, 1927. (i) Gordham, A. J.; Choyce, C. C., and Randall, M.: *Brit. J. Surg.* 16:62, 1928. (j) Pack, G. T., and Scharnagel, I.: *Am. J. Surg.* 58:369, 1928. (k) Walmsley, T.: *J. Anat.* 64:47, 1929. (l) Stewart, J. D.: *New England J. Med.* 203:1288, 1930. (m) Kline, L. B.: *Mil. Surgeon* 77:275, 1935.

REPORT OF CASE

A 29 year old white woman, married and a housewife, had always been in perfect health until eight months prior to operation, when intermittent cramp-like pains in the lower quadrant of the abdomen set in. There were no other symptoms. The menstrual history was not unusual. There was no fever at any time. The results of the physical examination were entirely negative except that some tenderness was found in the right lower quadrant of the abdomen.

After consultation with several physicians, Dr. I. W. Held advised an appendectomy. The patient was operated on Dec. 5, 1932. The postoperative course was uneventful. She has remained in good health to the present time, five years after operation.

Gross Description of Specimen.—The specimen was an appendix, approximately 9 cm. long. In the proximal half, it was normal in thickness. The tip was slightly club shaped and measured 2.1 cm. across. The fat tissue of the mesentery was adherent to the appendix and appeared normal. In the distal half, eight irregularly round and ovoid protrusions were seen in the serosa (fig. 1A). Five were situated close to each other, opposite or nearly opposite the attachment of the mesentery; the remainder appeared on the mesenteric border. The largest protrusion, which was cystic, measured 7 by 3 by 3 mm. Several of the smaller protrusions were opaque. There was no evidence of acute inflammation.

A longitudinal section of the appendix showed that the cavity of the aforementioned larger protrusion was in direct continuity with the lumen of the appendix (fig. 1A). This section, although passing nearly exactly through the middle, did not open a continuous lumen. In the 2 cm. near the tip, three small, irregularly round cavities were seen, one being continuous with the lumen of the larger cystic protrusion mentioned. It contained glassy mucoid material. These lesions corresponded to the protrusions found on the antimesenteric border of the appendix.

The three protrusions on the mesenteric border of the appendix, on closer outside examination, already were recognized as fat lobules. On the cut surface, they roughly overlay three solid polypoid herniations, 3.5 mm. apart and approximately 2 mm. in diameter. On this longitudinally bisected specimen the muscle coat was about 2 mm. thick and glassy gray. On the side of the solid protrusions, the cut edge was represented by partly round, partly elliptic markings separated from each other by strands of opaque grayish-white tissue. These strands continued in the direction of the mesentery, thus forming the solid polypoid herniations described. On the opposite side, the muscle coat was thicker and interrupted by two wide gaps, corresponding to the cystic protrusions (fig. 1B).

Microscopic Description.—The microscopic picture was as unusual as the gross. The larger cavity situated in one of the protrusions was a mucocoele, the tip of which was separated from the serosa of the appendix by only a thin layer of fibrosed submucosa. It contained much partly laminated mucoid material (the mucicarmine stain was positive).

The lumen of the appendix was narrow. The mucosa, as far as it was preserved, was not unusual. There were a moderate number of long and many more short glands. Goblet cells, for the most part, were few. Except for a sparsity of lymphocytes, the cellular pattern of the mucosa was not remarkable. The lymphatic follicles were few and small but otherwise normal.

The submucosa was thicker than normal, was rather densely fibrous and contained much more nerve tissue than one would expect.

The muscle coat was not continuous. It was separated into segments by the submucosal tissue, which protruded through wide gaps. Between the gaps, both muscular layers were seen. The round protruding masses of submucosal tissue

directly bordered on the serosa. Occasionally thin remnants of muscle tissue (visible only on higher magnification) were stretched over the convexity of the diverticula (fig. 2). The elastic tissue was preserved. The subserosa and serosa were not unusual.

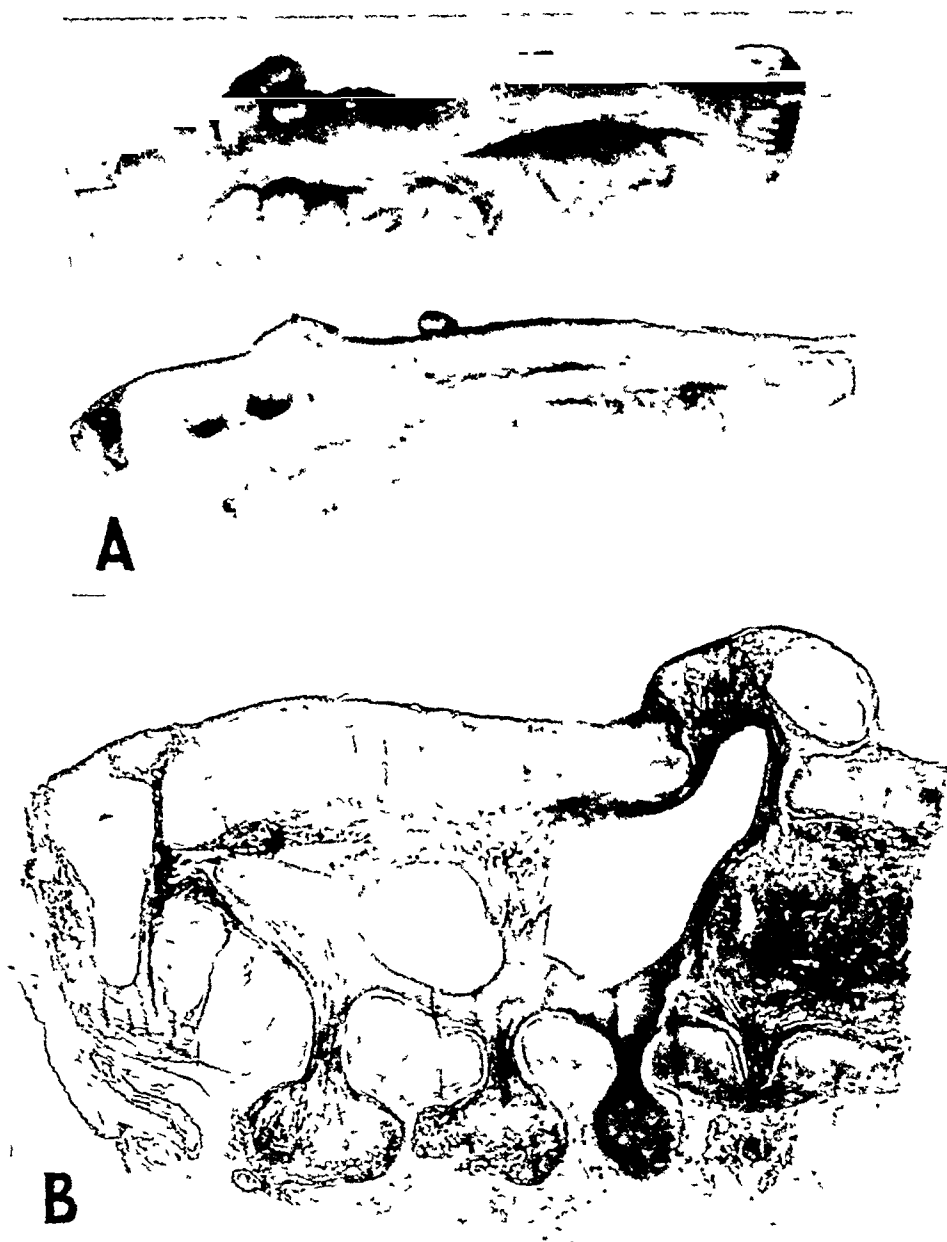


Fig. 1.—*A*, the exterior surface and a longitudinal section of the appendix, showing different types of protrusions and their relationship to the mesentery. *B*, the long section under very low magnification. In the central upper portion, the muscle coat appears unbroken. To the right and left, diverticula are seen. Their mucosal lining cannot be recognized at this magnification. In the lower portion, the muscle coat is broken up. Dense masses of submucosa reach through wide gaps in the muscle and spread in polypoid fashion. They are surrounded by fat tissue. At the right lower corner, the section has gone obliquely through the narrow pedicle of one protrusion.

The solid protrusions consisted essentially of submucosal connective tissue with many blood vessels and an enormous amount of nerve tissue (fig. 2). The nerve tissue predominated to such an extent that in the sections stained by Van Gieson's method it could be seen with the naked eye. There was no overgrowth of ganglion cells.

The inside of each cystic protrusion was mostly lined by a single layer of columnar cuboidal or flattened epithelial cells, under which some traces of lymphoid tissue were found. In the mucocoele-like portion, no mucosa was seen. The mucus had separated the innermost layers of the surrounding wall.



Fig. 2.—High magnification of a solid protrusion, showing the presence of neuromas and diffuse hyperplasia of nerve tissue. Thin remnants of muscle surround the diverticulum. Note the overgrowth of nerve tissue in the vascular connective tissue of the submucosa.

COMMENT

Although new interest in this lesion has recently appeared, diverticula of the vermiform appendix probably occur more commonly than one would judge from the available reports.⁵¹ No doubt many more cases would be recognized were a longitudinal section of the appendix,

passing through the mesenteric border,⁶ or many cross sections examined. Another factor responsible for the low reported incidence is the failure of this condition to produce clinical symptoms.

Yet one should not minimize the complications that may ensue. When acute inflammation of the appendix sets in, rapid perforation of the thin-walled diverticula may occur after only a few hours of vague abdominal symptoms.^{4e} A review of the surgical literature gives percentages from 39^{4c} to 100^{4b} for the occurrence of acute inflammation in appendixes that are the seat of diverticula. These figures, no doubt, would be much lower were the appendixes in which diverticula were found accidentally at autopsy included in these statistics. When the diverticula are associated with mucocoele, as in the present case, perforation with the development of pseudomyxoma peritonei is not an unusual finding.⁵¹ Diverticula of the vermiform appendix occasionally have perforated into other viscera, with the formation of fecal fistulas.^{4d}

The theories regarding the genesis of the multiple diverticulum are almost as numerous as the observers reviewing the subject. Weakness of the muscular wall, either congenital or acquired, plus increased internal pressure, was mentioned at one time as the causative factor.⁷ Mertens⁸ observed that diverticula pass through arterial gaps in the muscular wall of the appendix. Aschoff⁹ and later Chase^{5h} held that the loss of fat and fibrous tissue about these vascular gaps could cause definite weakness at a point later the site of a diverticulum. Mulsow^{4d} maintained that continuous traction from without, as by adhesions, may ultimately produce diverticula.

The role of antecedent inflammation in the genesis of diverticula has been emphasized by many observers.¹⁰ Some maintain that it is the sole etiologic agent.¹¹ While one cannot deny that a preceding intramural abscess with destruction of the muscularis, followed by healing and cicatrization, may leave a locus minoris resistentiae at which herniation of the submucosa may occur, one is not justified in assuming that it is the only underlying factor. For surely, in many of these appendixes, even a careful study fails to reveal recent or old inflammation. In the case reported here and in 6 others collected over nine years at the Beth Israel Hospital, no inflammation was found. The lumen of the appendix in the specimen described in this report was uniformly narrow, but no local point of obstruction was encountered.

6 Moschcowitz.^{4b} Stout.^{4c}

7. Heschl: Wien. med. Wchnschr. **30**:540, 1880. Beer, E. H.: Am. J. M. Sc. **128**:135, 1904.

8. Mertens, H.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **9**:743, 1902.

9. Aschoff, L.: Spezielle pathologische Anatomie, in Pathologische Anatomie: Ein Lehrbuch für Studierende und Aerzte, ed. 3, Jena, Gustav Fischer, 1913, vol. 2, p. 824.

10. Moschcowitz.^{4b} Edwards.^{4e} Sauer.^{4f} Collins.^{4g}

11. Mundt, T.: Ueber Veränderungen der Muskelwand des Wurmfortsatzes, in Pathologische-anatomische Arbeiten: Herren Geh. Medicinalrath Dr. Johannes Orth zur Feier seines 25jährigen Professor-Jubiläums, Berlin, A. Hirschwald, 1903, pp. 463-470. Moiroud, P., and Imbert, R.: Bull. et mém. Soc. nat. de chir. **58**:732, 1932. Moschcowitz.^{4b} Edwards.^{4e}

Christeller¹² subdivided diverticula of the appendix into two categories, namely, those at sites of previous inflammation and not caused by mechanical factors, such as narrowing of the lumen and increase of internal pressure, and those caused by mechanical factors such as those mentioned. In the latter group, the narrowing may be due to postinflammatory scarring or to a noninflammatory process. The case I have reported would fit into the latter classification, the narrowing being unrelated to any inflammatory changes.

Stout^{4c} suggested that the contractions of the circular and longitudinal muscle coats are the chief active factors in driving mucosa and submucosa through a weakened point in the muscularis. In a series of controlled experiments on the dog's appendix he found that operative muscular defects resulted immediately in protrusion of mucosa and submucosa. This was accompanied by active contractions of the circular and longitudinal muscles. When the muscles had lost their contractility, no protrusions occurred provided the lumen remained patent. The experimental defects were much larger than the corresponding spontaneous defects in the human patient, and certainly they were formed much more rapidly.

As previously mentioned, none of the case reports contains any description of the neuromatous change in the diverticula.¹³ Neuroma of the appendix is much more frequent than most physicians realize. In the material at the Beth Israel Hospital, every second appendix examined (when a sufficient number of sections is taken) shows neuroma. Other authors give similar, some even higher, figures. Of 344 consecutive appendixes examined by Hosoi,¹⁴ 195 (56.7 per cent) showed nerve lesions. In a similar study Simard¹⁵ found that neuroma occurs in 75.2 per cent of obliterated appendixes. Fein, Hanan and Seider¹⁶ reported 202 neuromas in a series of 600 consecutive appendixes (an incidence of 33 per cent).

In view of the obscure etiologic background of both the diverticula and the neuroma, it is not astonishing that I am unable to explain their simultaneous occurrence in this specimen. As long as the problem of "chronic appendicitis" remains unsolved, one cannot expect to understand the possible connection between chronic inflammatory processes in the appendix and the occurrence of herniation through the vascular gaps. Moreover, none of the theories given in the literature, in my opinion, adequately explains the formation of diverticula of the vermiform appendix.

12. Christeller, E., in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1929, pt. 3.

13. A recently described case (Wunder, E.: *Frankfurt. Ztschr. f. Path.* **51**: 18, 1937) bears a certain similarity to the one I have reported except for the fact that no neuromatous change is mentioned. It concerned a 39 year old man who had vague abdominal pain, tending to localize in the right lower quadrant. In the appendix, partly cystic, partly solid diverticula were found.

14. Hosoi, E.: *Arch. Path.* **16**:500, 1933.

15. Simard, L. C.: *Canad. M. A. J.* **33**:518, 1935.

16. Fein, M. J.; Hanan, J. T., and Seider, V. B.: *Am. J. Surg.* **40**:120, 1938.

SUMMARY

In the appendix of a 29 year old woman who had presented vague abdominal symptoms, 8 partly cystic, partly solid diverticula were found. One of the cystic protrusions gave the picture of a partial mucocele. A diffuse neuromatous lesion occupied most of the submucosa in the solid diverticula.

No record of a similar case could be found in the literature.

COARCTATION OF THE AORTA OF THE ADULT TYPE, ASSOCIATED WITH CYSTIC DEGENERATION OF THE MEDIA IN THE FIRST PORTION OF THE ARCH

F. F. HARRISON, M.D., COOPERSTOWN, N. Y.

Coarctation of the aorta is not a particularly unusual congenital cardiovascular anomaly. According to autopsy statistics collected by Blackford,¹ it occurs once in about 1,550 cases. The present case seems worthy of note, however, because of the fact that during the patient's lifetime it was carefully studied and reported from a physiologic standpoint² and also because the microscopic observations in the aorta are of considerable interest.

REPORT OF CASE

A medical student 27 years of age was admitted to the hospital June 11, 1935. Eleven years previously he had been found to have a systolic blood pressure of 185 mm. and signs of endocarditis. The correct diagnosis, however, was not made until his entrance into medical school in 1931. At this time roentgen examination of the chest showed scalloping of the inferior borders of the ribs, and there were characteristic signs of coarctation of the aorta, together with an associated aortic insufficiency. The condition was then well compensated. Two years later, in collaboration with Grollman² he published a study of his own cardiac output. The final illness began with an infection of the upper part of the respiratory tract three weeks before admission. An exacerbation of this infection four days before admission initiated a rapid break in cardiac compensation.

The temperature was 102 F.; the pulse rate, 120; the respiratory rate, 24. The blood pressure in the right arm was 148 systolic and 70 diastolic; that in the left arm, 180 systolic and 70 diastolic; that in the legs, 98 systolic and 70 diastolic.

The patient appeared desperately sick, with marked pallor, diaphoresis and extreme dyspnea. The heart was tremendously enlarged, with loud systolic and diastolic murmurs. There were dulness, increased breath sounds and moist rales at the base of the left lung. The laboratory findings were all essentially negative. A roentgenogram showed scalloping of the inferior margins of the ribs and a huge heart. He died in less than forty-eight hours after admission.

Autopsy.—The panniculus was scanty; the musculature, well developed and deep red. The superior epigastric arteries were unusually large and apparently anastomosed with the internal mammary and inferior hypogastric arteries. The lower border of the liver lay 11 cm. below the xiphoid process and 5 cm. below the right costal margin in the nipple line. The spleen was somewhat enlarged but was well above the costal margin.

From the Department of Pathology, Mary Imogene Bassett Hospital.

1. Blackford, L. M.: Arch. Int. Med. **41**:702, 1928.

2. Grollman, A., and Ferrigan, J. P.: Arch. Int. Med. **53**:35, 1934.

Both lungs were collapsed and lay well back in each side of the thoracic cage. There was no free fluid in either pleural cavity. The pericardial cavity contained the usual amount of straw-colored fluid.

The heart and the aorta weighed 932 Gm. The epicardial surface was smooth. There was a fibrous tag near the apex of the heart. All four chambers of the heart were widely dilated and filled with clotted blood. The right and left anterior aortic leaflets showed some thickening and rolling downward of the free edges, and on the right anterior leaflet there was a small gelatinous-appearing nodule, possibly 1 mm. in diameter. The wall of the right ventricle measured 8 mm. in thickness; that of the left, 26 mm. The right coronary artery had a double origin, one component of which was small.

The circumference of the aorta was found to be as follows:

	External	Internal
At attachment of cusps.....		12 cm.
6 cm. above cusps.....	8.5 cm.	7 cm.
At innominate artery.....	7 cm.	6.5 cm.
Between left carotid and left subclavian arteries	4.5 cm.	4 cm.
Just beyond left subclavian artery.....	4 cm.	3.5 cm.
At coarctation	3.2 cm.	0
At celiac axis	4.5 cm.	3.7 cm.
At bifurcation of aorta.....		2.0 cm.

The ascending part of the aortic arch was widened into an oval-shaped bulbous enlargement. This narrowed rapidly at a point 4 cm. above its origin, and then gradually until the coarctation was reached. The narrowest point of the aorta was about 7 mm. beyond a nonpatent fibrous cord representing the ductus arteriosus.

There was a slight amount of atheroma of the ascending aorta, which became more marked just above the coarctation. No lumen could be demonstrated at the point of coarctation, and just below it the atheroma was again only slight. The aorta beyond this point continued to be markedly narrowed as indicated in the measurements.

The collateral circulation of the occluded aorta seems to have been accounted for largely by the internal mammary, transverse cervical and transverse scapular arteries. The first-named vessels were very large, the lumen before fixation being nearly 5 mm. in diameter. The intercostal branches from the internal mammary arteries were small down to the fifth interspace. In the fifth and sixth interspaces large branches were given off. Below this point the vessels passed into the substance of the rectus muscle, where an anastomosis was formed with the deep epigastric artery.

The right lung weighed 425 Gm.; it was crepitant throughout; the parenchyma was rusty brown and quite dry.

The left lung weighed 350 Gm. Its general appearance was much like that of the right. The whole lower lobe felt somewhat boggy, and over the posterior aspect of the pleural surface a small amount of fibrin was deposited. At the inferior anterior border of this lung, in about the anterior axillary line, there was a large infarct, measuring about 8 by 6 cm. in its greatest diameters. On section there was a little more moisture in this lower lobe than in its fellow, but no actual areas of consolidation were made out.

The spleen weighed 260 Gm. In the hilus were two accessory spleens. The organ presented gross evidences of chronic passive congestion.

The liver together with the gallbladder weighed 1,925 Gm. They appeared grossly normal.

The stomach, intestines, pancreas and adrenals also appeared grossly normal. Each kidney weighed 175 Gm. In the midportion of the left kidney was a small infarct, and the capsule stripped with some difficulty.

The pelvis, ureters and bladder presented no abnormalities. The genitalia appeared grossly normal.

Blood cultures showed no growth after incubation for ten days.

Microscopic Examination.—The individual myocardial fibers showed marked hypertrophy. The sections were studded with small areas in which an increase in

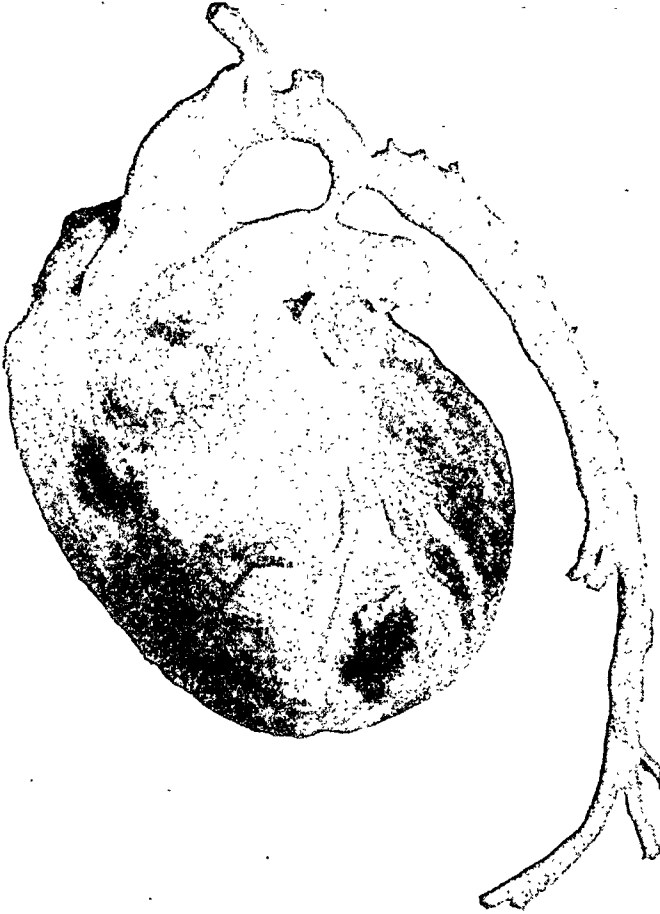


Fig. 1.—Heart and aorta.

fibrous connective tissue was apparent. This change was particularly notable in the immediate vicinity of the smaller branches of the coronary arteries.

The nodule on the end of the right anterior cusp of the aortic valve was bordered by dense connective tissue, within which there was a zone of less dense, rather myxomatous-looking tissue, containing two more or less rounded masses of acellular material. There was no evidence of a recent inflammatory reaction, and no organisms could be demonstrated with bacterial stains.

Sections through the first portion of the aorta showed well marked changes characteristic of atherosclerosis. A considerable amount of calcium was deposited in the half of the media adjacent to the intima. Deeper in the media many fibers appeared swollen, and often they were fragmented, with cystic areas between them, usually filled with mucinous-looking material. There was practically com-

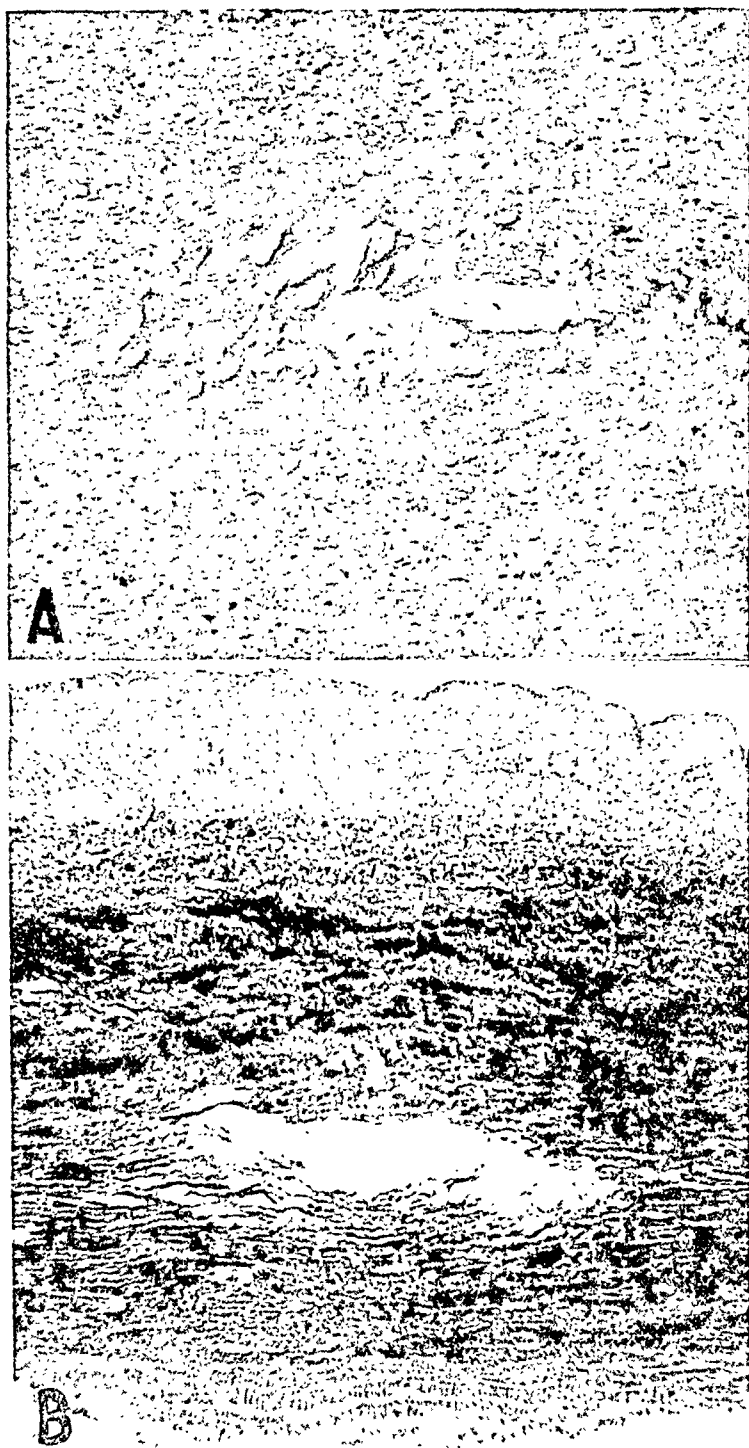


Fig. 2.—*A*, first portion of the aorta, showing a cystic area in the outer half of the media. Hematoxylin-eosin stain; $\times 250$. *B*, first portion of the aorta, showing cystic area in outer half of the media; Mallory's aniline blue stain; $\times 150$.

plete absence of cellular reaction in these areas. With the aid of stains for elastic tissue one obtained the impression that one of the earlier events was swelling and dissolution of collagen fibers, the elastic fibers at first remaining intact. Later, as the cavity thus created grew larger, the broken ends of the elastic fibers protruded into it. Still later they lay as fragments in the midst of the myxomatous material and eventually disappeared. At several points well preserved nuclei of smooth muscle could be demonstrated in the substance of the pronglike projections into the cystic cavities.

A section of aorta taken from a point near the origin of the left subclavian artery showed nothing unusual other than a moderate deposition of calcium in the media. In a block taken 1 inch (2.5 cm.) below the point of coarctation, however, rather marked atheromatous intimal changes were again apparent.

The sections of lung showed marked chronic passive congestion. One section taken from the infarcted area showed typical obliteration of the architecture with great numbers of red cells.

Sections of the spleen showed evidences of chronic passive congestion. The liver showed well marked evidence of chronic passive congestion and, in addition, a moderate amount of fatty infiltration in the midzonal region. Only the liver cells immediately adjacent to the portal spaces appeared normal. The kidneys and adrenals showed nothing unusual other than congestion.

The thyroid gland showed a moderate increase in connective tissue stroma. There was no evidence of activity of the gland.

Anatomic Diagnosis.—The following conditions were observed: coarctation of the aorta of the adult type, with complete atresia of the lumen; fibrosis of the coronary cusps of the aortic valve, with insufficiency; cardiac hypertrophy and dilatation; atheroma of the aorta; cystic medial degeneration of the aorta; acute fibrinous pleuritis; infarct of the lower lobe of the left lung and of the left kidney; general chronic passive congestion of the viscera; fatty infiltration of the liver.

COMMENT

Aneurysm of the aorta, almost always involving the first portion, is not uncommonly associated with coarctation and is frequently the cause of death. The descriptions of the histologic picture in the aorta have been most thorough in the cases in which rupture occurred. Bronson and Sutherland³ reported an instance of coarctation with rupture of a fusiform aneurysm into the pericardium in a boy of 5. They described the degenerative changes as occurring predominantly in the outer part of the intima and the subjacent inner half of the media. They assumed that there had been two factors: (1) a congenital defect in the wall and (2) so-called protective sclerosis, resulting from the increased tension.

Blackford¹ held that the occurrence of rupture of the aorta in some cases in which stenosis was slight lends support to the idea that there existed in these instances an imperfect development of the wall of the aorta. On the other hand, the fact that in the greatest number of instances rupture occurred during or immediately subsequent to strenuous exertion tends to emphasize the factor of mechanical strain. A case in which mechanical strain could not have been a factor is that reported by Smith and Targett.⁴ In this instance the aneurysmal

3. Bronson, E., and Sutherland, G. A.: *Brit. J. Child. Dis.* **15**:241, 1918.

4. Smith, F. J., and Targett, J. H.: *Tr. Path. Soc. London* **48**:53, 1896-1897.

opening was situated one-half inch beyond the coarctation. They described a thinning of the wall with a disproportionate loss of elastic fibers at and just above the coarctation. At the aneurysm there was an abrupt rupture of elastic fibers, with fraying of the broken ends.

Medial degeneration of the type found in my case is generally conceded to be the most common cause of dissecting aneurysm. An excellent report of cases of this type was published by Tyson.⁵ The report includes 5 cases. One was probably a case of aneurysm due to syphilis. Marked degenerative changes in the media were found in the ascending aorta in all 5 cases. Associated with these changes was marked intimal thickening of the vasa vasorum in the adventitia. The most detailed descriptions of nonsyphilitic degenerative disease are those in the two papers published by Erdheim.⁶ He emphasized the fact that in the disappearance of tissue the elastic fibrils were most conspicuously involved, the connective tissue less and muscle least. Great stress was laid on the absence of cellular exudate—a notable feature in my case as well. In contrast with Tyson,⁵ Erdheim dismisses the possibility that disease of the vasa vasorum is an etiologic factor.

In the present instance it is quite possible that the association of coarctation with cystic degeneration of the media may have been wholly fortuitous. On the other hand, this association raises the question whether rupture of the aorta, which occurs with fair frequency in cases of coarctation, may not often have such a lesion as its pathologic basis.

SUMMARY

A case of coarctation of the aorta of the adult type is reported in a man who died at the age of 27 of rapidly developing cardiac failure. Associated with the coarctation was not only disease of the aortic valves but also dilatation and cystic medial degeneration of the first portion of the aorta.

5. Tyson, M. D.: *Am. J. Path.* **7**:581, 1931.

6. Erdheim, J.: *Virchows Arch. f. path. Anat.* **273**:454, 1929; **276**:187, 1930.

COMPLETE ANURIA DUE TO BLOCKAGE OF RENAL TUBULES BY PROTEIN CASTS IN A CASE OF MULTIPLE MYELOMA

RUSSELL L. HOLMAN, M. D., CHAPEL HILL, N. C.

Disturbances in renal function associated with excretion of protein in cases of multiple myeloma have been commented on by numerous observers,¹ but so far as I am aware no case of complete anuria due to this condition has been reported. None of the standard references consulted² lists anuria as a possible complication of multiple myeloma. The case reported here is of interest because of the extreme degree of obstruction of the renal tubular system by protein casts. Both the clinical data and the histologic appearance of the kidneys indicated that the precipitation of the abnormal protein occurred extensively within a relatively short period of time. The view expressed recently by Forbus, Perlzweig, Parfentjev and Burwell^{1b} that the damage associated with excretion of Bence Jones protein is due primarily to mechanical obstruction and not to any specific toxic effect on the tubular epithelium is supported by this case.

A colored housemaid aged 43, married, entered the Presbyterian Hospital, New York, on May 4, 1936, complaining of pain in the right thigh of three weeks' duration. The familial and past histories were irrelevant. She had not menstruated for seven months. For three months there had been progressively increasing fatigue, weakness, insomnia and anorexia, with loss of 32 pounds (14.5 Kg.) in weight. Three weeks before admission pain began in the region of the right hip and increased in severity until walking became unbearable.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

1. (a) Decastello, A.: *Ztschr. f. klin. Med.* **67**:319, 1909. (b) Forbus, W. D.; Perlzweig, W. A.; Parfentjev, I. A., and Burwell, J. C., Jr.: *Bull. Johns Hopkins Hosp.* **57**:47, 1935. (c) Groat, W. A., and Brewer, R. K.: *J. Lab. & Clin. Med.* **1**:895, 1916. (d) Hopkins, F. G., and Savory, H.: *J. Physiol.* **42**:189, 1911. (e) Jacobson, V. C.: *J. Urol.* **1**:167, 1917.

2. Allbutt, T. C., and Rolleston, H. D.: *System of Medicine*, London, Macmillan & Co., 1909. Christian, H. A., and Mackenzie, J.: *Oxford Medicine*, New York, Oxford University Press, 1936. French, H.: *Index of Differential Diagnosis*, Baltimore, William Wood & Company, 1935. Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1930. Kaufmann, E.: *Pathology for Students and Practitioners*, translated by S. P. Reimann, Philadelphia, P. Blakiston's Son & Co., 1929. Osler, W., and McCrae, T.: *Modern Medicine*, New York, Lea Bros. & Co., 1907. Nelson Loose-Leaf Living Medicine, New York, Thomas Nelson & Sons, 1937. Tice, F.: *Practice of Medicine*, Hagerstown, Md., W. F. Prior Company, Inc., 1922.

Laboratory Data on Blood and Urine

Date (1920)	Blood															
	Hemo- globin Per- centage (Sahlb)	White Blood Cells				Serum			Fluids		Urine					
		Total Count	Poly- morpho- nuclears, per Cent	Mono- cytes, per Cent	Pro- tein, Mg. in 100 Cc.	Albu- min, Mg. in 100 Cc.	Nonprotein Nitrogen, Mg. in 100 Cc.	Daily Aver- age Intake, Cc.	Daily Aver- age Out- put, Cc.	Reac- tion	Specific Gravity	Albu- min	Casts	Red Blood Cells per High Power Field	White Blood Cells Loaded	Bence- Jones Protein
May 4-17	62	3.3	5,600	25	75	8.0	0.7	29	Not recorded	Acid	1.025	+	Few	2.5	Loaded
May 18-31	49	2.7	8,100	50	50	Not recorded	Acid	1.020	+	Few	1-2	Loaded	None
June 1-11	45	2.2	3,650	61	39	Not recorded	Neutral	1.015	++	Many	0	Few
June 15-23	46	2.2	3,800	73	27	Not recorded	Acid	1.012	+++	Many	8	Loaded
June 29 to July 12	44	2.5	2,900	59	41	6.4	1.0	150	1,600	Alkaline	1.010	+++	None	3.8	Many	None (2 ex- aminations)
July 14	26	1.3	170	2,000	Acid	Quantity insufficient	+	None	0	4 per high power field	None
July 17	24	0.9	1,200	69	31	0	0	0	0	0	0	0	0
July 22	21	1.1	1,200	63	37	167	0	0	0	0	0	0	0	0

Complete anuria since July 14

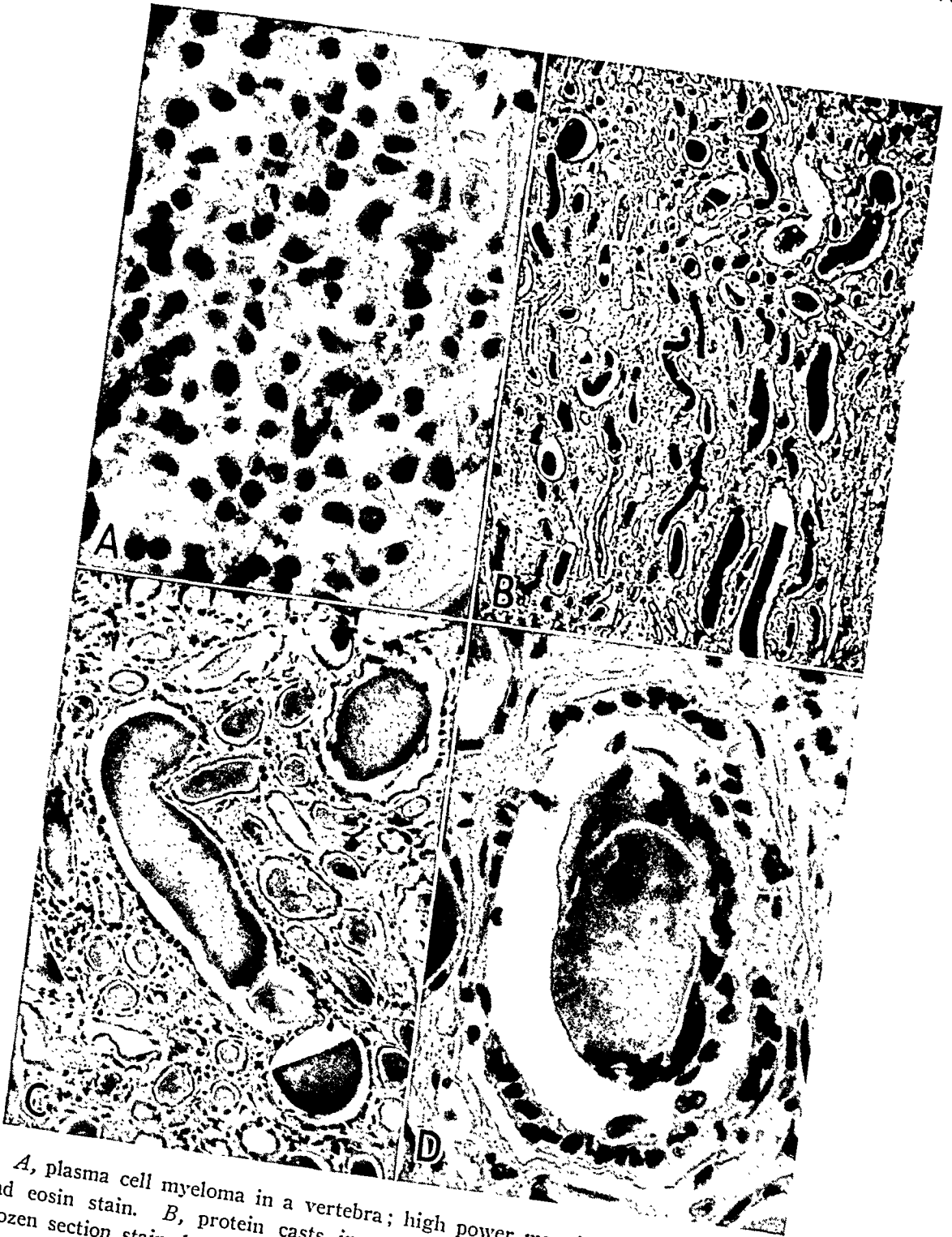
The temperature was 99.4 F.; the pulse rate 90, the respiratory rate, 18; the blood pressure, 102 systolic and 66 diastolic. No other abnormality was noted save the evidence of loss in weight and the pain on hyperextension of the right thigh. The laboratory findings pertaining to the blood and urine are summarized in the table. The Wassermann reaction of the blood was negative, and gastric analysis gave negative results. The erythrocyte sedimentation rate was from 80 to 164 mm. in an hour. The reticulocyte percentage on June 9 was 1. The bleeding time on July 5 was 12 minutes. Roentgenograms of the gastrointestinal tract, made with the aid of a barium sulfate enema, revealed no abnormality. Roentgenograms of the right leg, pelvis, spine, chest and sinuses on May 5 disclosed no lesions.

From the time of admission the patient presented a diagnostic problem. The first intimation of an organic lesion appeared in a roentgenogram of the spine, right hip and thigh taken on June 1; this showed an area of diminished density in the neck of the right femur. Similar lesions were seen later in the tenth dorsal and some of the lumbar vertebrae. Cystoscopic and pyelographic examinations on June 12 revealed no abnormality. Roentgen rays of high voltage were directed to the right hip in a course of treatments started on June 16; there was occasional vomiting following these treatments. Oliguria occurred from July 2 to July 14 and anuria from July 15 to July 23, the cause of which was never discovered. The patient's bladder was catheterized on July 13, but only a few cubic centimeters of urine was obtained. Parenteral injections of saline and dextrose solutions were started on July 5, and the amounts injected were increased until as much as 4,350 cc. was being given on July 13. These injections were discontinued after July 15. With the administration of the fluids mentioned, the patient became edematous and—as can be seen from the table—hydremic. The nonprotein nitrogen of the blood increased rapidly to 167 mg. in 100 cc. The patient died in uremia on July 23. No definite clinical diagnosis was made; suggestions were (1) lymphoblastoma, site unknown; (2) Hodgkin's disease, site unknown; (3) multiple myeloma.

Necropsy was performed twenty-two and one-half hours post mortem. The resultant diagnoses other than those directly related to the kidneys were: multiple myeloma (plasma cell type) involving vertebrae (ninth and tenth dorsal and third lumbar as shown in A in figure), the sternum, the neck of the right femur and the left parietal pleura; secondary myeloma in the liver; aplasia of the femoral bone marrow; hemosiderosis and extramedullary erythropoiesis in the spleen and liver; fatty infiltration of the heart; edema of the lungs; bilateral hydrothorax; ascites; edema of the hands and eyelids; acute and chronic cystitis; myomas of the uterus.

The kidneys were identical in appearance. Each weighed 180 Gm. The capsule stripped easily, disclosing a very pale gray surface. The cut surface was also pale gray. The cortex was of uniform width and measured 0.6 cm.; the cortical striations, though blurred, could be made out. The pyramids were sharply defined, and the pyramidal striations were distinct. The blood vessels appeared normal. The mucosa of the renal pelvis was slightly congested. The ureters were normal. In the bladder were only a few cubic centimeters of slightly turbid yellow urine.

Histologic examination disclosed that all the loops of Henle, the distal convoluted tubules and the collecting tubules were filled with dense hyaline casts (B in figure); about the margins of some of these were foreign body giant cells (D in figure) similar to those pictured by Forbus and his co-workers and claimed by them to be "specifically indicative of Bence-Jones proteinuria." The glomeruli appeared normal except for distention of the spaces of Bowman and slight thicken-



A, plasma cell myeloma in a vertebra; high power magnification; hematoxylin and eosin stain. *B*, protein casts in renal tubules; low power magnification; frozen section stained with sudan III. *C*, distention of tubules by casts; medium power magnification; hematoxylin and eosin stain. *D*, foreign body giant cell about a protein cast in a renal tubule; high power magnification; hematoxylin and eosin stain.

ing of the capsular basement membranes. The proximal convoluted tubules were dilated and the lining epithelium flattened. The remaining portions of the tubules were also slightly dilated; in some of them the cast distended the lumen to more than twice its normal diameter (*C* in figure). These tubules were lined by flattened epithelium. The interstitial tissue was edematous and here and there infiltrated by large mononuclear cells in clusters. Some of these cells resembled myeloma cells seen elsewhere but could not be definitely identified as such. The blood vessels were normal. The epithelial cells of the pelvic mucosa had desquamated; the basement membrane was everywhere intact. In the underlying stroma were groups of cells similar to those described between the tubules. There was no exudate on the mucosal surface of the urinary bladder. The mucosal stroma was edematous and congested and was diffusely infiltrated with mononuclear and polymorphonuclear leukocytes. No organisms were seen in a section stained by Gram's method.

COMMENT

As can be seen from the photomicrographs, the essential lesion was a typical myeloma of the plasma cell type. The chief interest in the case is the effect produced on the kidney by the abnormal protein being excreted. The only proof that this protein was of the Bence Jones variety was the presence of giant cells of the foreign body type about some of the casts in the renal tubules.^{1b} The urine had been tested for this protein on four occasions, but none was found. It is well known, however, that the excretion of Bence Jones protein may be intermittent and that this protein is not found in the urine in every instance of multiple myeloma. Whatever name may be applied to the protein in the urine of this patient, it is obvious that one of its peculiar properties was coagulability. The protein apparently passed through the glomeruli without leaving histologic evidence of damage and first became solidified in the loops of Henle or more distal parts of the tubular system. Whether this was a consequence of reabsorption of water in the proximal convoluted tubules, with resultant increase in the concentration of the protein, or whether it was related to a change in the concentration of hydrogen ions or of electrolytes^{1c} in the urine is conjectural. The result of the deposition of this protein was a mechanical blockage of the tubular system. The absence of detectable changes in the glomeruli with the dilatation of the spaces of Bowman and of the proximal convoluted tubules further supports the view that the damage was due to mechanical obstruction and not to any specific toxic effect of the abnormal protein on the tubular epithelium.

SUMMARY

A colored woman of 43 years died in uremia after twelve days of marked oliguria, followed by nine days of complete anuria. Terminally the nonprotein nitrogen of the blood rose to 167 mg. in 100 cc. All the renal tubules beginning with Henle's loop were plugged with hyaline casts. About the margins of some of these casts were foreign body giant cells. The proximal convoluted tubules and the spaces of Bowman were dilated. Since no other cause for the anuria could be found, it is assumed that it resulted from the mechanical obstruction.

Dr. Walter Palmer gave me permission to abstract the clinical data on this case.

General Reviews

HYPERPARATHYROIDISM AND RENAL DISEASE .

W. A. D. ANDERSON, M.D.

MEMPHIS, TENN.

As knowledge of the function and of abnormal conditions of the parathyroid glands has rapidly increased in recent years it has become evident that there is a close linkage of these glands with renal function and disease. It is now known that lesions in the skeletal system are not the only important changes associated with hyperparathyroidism. In many cases renal disease may overshadow the skeletal changes. MacCallum¹ first drew attention to this relationship in 1905, when he reported a tumor of a parathyroid gland associated with chronic renal disease. Since that time many scattered reports have indicated a relationship between hyperparathyroidism and disease of the kidney. Much of the credit for knowledge of this subject must be given to Albright and his associates² for their numerous careful studies on this phase of hyperparathyroidism. Considerable confusion of terminology and etiology has resulted, however, partly because investigators are still not certain of the stimuli, normal or pathologic, which induce parathyroid secretion or of the mechanism of the action of this secretion.

In the present review an attempt will be made to survey reported studies which have a bearing on the problem of a relationship between increased parathyroid function and renal disease. It is hoped that a marshaling together of various case reports, experimental studies and

From the Department of Pathology, University of Tennessee College of Medicine.

1. MacCallum, W. G.: *Bull. Johns Hopkins Hosp.* **16**:87, 1905.

2. (a) Albright, F.: *M. Clin. North America* **18**:1109, 1935; (b) *New England J. Med.* **217**:1063, 1937; (c) *Tr. A. Am. Physicians* **51**:199, 1936; (d) **52**:171, 1937; (e) *Internat. Clin.* **3**:280, 1937. (f) Albright, F.; Aub, J. C., and Bauer, W.: *J. A. M. A.* **102**:1276, 1934. (g) Albright, F.; Bauer, W.; Cockrill, J. R., and Ellsworth, R.: *J. Clin. Investigation* **9**:659, 1931. (h) Albright, F., and Bloomberg, E.: *Tr. Am. A. Genito-Urin. Surgeons* **27**:195, 1934; (i) *J. Urol.* **34**:1, 1935. (j) Albright, F.; Baird, P. C.; Cope, O., and Bloomberg, E.: *Am. J. M. Sc.* **187**:49, 1934. (k) Albright, F.; Bloomberg, E.; Castleman, B., and Churchill, E. D.: *Arch. Int. Med.* **54**:315, 1934. (l) Albright, F.; Drake, T. G., and Sulkowitch, H. W.: *Bull. Johns Hopkins Hosp.* **60**:377, 1937. (m) Albright, F.; Sulkowitch, H. W., and Bloomberg, E.: *Am. J. M. Sc.* **193**:800, 1937; (n) *Arch. Int. Med.* **62**:199, 1938.

theories may clarify research in this subject, point out gaps in knowledge and suggest lines along which future work may be profitable. As far as is consistent with intelligent consideration of the subject only those aspects of hyperparathyroidism which are directly related to renal function and disease will be considered.

Two facts stand out and at first seem confusing: 1. Primary renal disease may result in parathyroid hyperplasia and hyperfunction, the degree and effects of which are modified in individual cases by the severity of the renal deficiency and by factors influencing the metabolism of calcium and phosphorus.

2. Hyperparathyroidism, whether arising primarily in a functioning neoplasm of the parathyroids or secondarily in response to some stimulus from elsewhere, may produce renal damage varying from slight to severe also modified by other factors in addition to the severity of the hyperparathyroidism. In these relationships disturbances in the metabolism of calcium and phosphorus play a prominent part.

RELATIONSHIPS BETWEEN PARATHYROID AND RENAL FUNCTIONS

It has long been established that the parathyroid glands play an essential part in the metabolism of calcium and phosphorus and regulate their concentration in the serum. The point of action of the parathyroid hormone in the mechanism of this regulation has been a matter of some dispute. Approximately half of the normal 9 to 11 mg. of calcium in 100 cc. of serum is held in an inactive nondiffusible compound, bound in some way to serum protein. The remaining ionized portion is normally maintained within a relatively narrow range by parathyroid regulation. Fluctuation in the serum protein may cause considerable variation in the total serum calcium without the ionized, parathyroid-regulated portion varying beyond normal limits. Conversely, determinations of the total calcium may reveal no abnormality although the concentration of the ionized fraction is such as to indicate considerable disturbance of parathyroid function. The concentration of calcium ions in the plasma at any one time is the resultant of an equilibrium between the total calcium and the total protein present in the plasma. It is thus evident that, for significance regarding the parathyroids, any determination of serum calcium must take into account the level of plasma proteins. An increase in the concentration of phosphates in the serum may bring about a lowering of the ionized calcium. In hypoparathyroidism and hyperparathyroidism serum calcium and phosphorus maintain a relationship such that their product remains roughly constant except at high levels of serum calcium, when the phosphorus may rise abruptly. A third factor of importance is the alkalinity of the serum.

Albright,^{2c} Goadby and Stacey³ and Ellsworth and others^{4a, b} noted that one of the early and prominent effects of injection of parathyroid extract is a phosphate diuresis. This increased excretion of phosphates is independent of the concentration of inorganic phosphorus in the blood plasma. Goadby and Stacey^{3a} pointed out that there are three possibilities as to the point of action of the parathyroid hormone: (1) the mechanism of the kidney's excretion of inorganic phosphates, (2) the renal phosphatase and (3) the inorganic phosphorus of the blood or tissues. Their later investigations showed that in patients with severe impairment of renal function the phosphate diuresis was impaired or failed to appear. On improvement of renal function—as after recovery from an attack of acute nephritis—the phosphate diuresis again increased. It thus appears that the parathyroid hormone may act directly on the kidneys to allow increased excretion of phosphorus, resulting in a lowering of the serum phosphorus and a reciprocal rise in serum calcium. The matter would be simplified if it were not for evidence of other effects of the parathyroid hormone. However, Thomson and Pugsley,⁵ Selye⁶ and Thomson and Collip⁷ found that the parathyroid hormone may directly stimulate osteoclasts to mobilize calcium from the bones and that this characteristic skeletal effect may be obtained even after bilateral nephrectomy. McJunkin, Tweedy and McNamara⁸ also showed this characteristic osteoclastic reaction after bilateral nephrectomy but found that the increase of serum calcium which usually accompanies injection of parathyroid extract was not obtained. They suggested that in the nephrectomized animal, in which the serum phosphorus rises, the mobilized calcium does not accumulate in the blood because of increased elimination by the intestinal route. Ellsworth and Futcher,^{4b} on the other hand, were able by injection of parathyroid extract to obtain increased serum calcium in bilaterally nephrectomized animals.

While this seems rather confusing, it appears to establish that there are at least two points at which parathyroid extract may have an effect on calcium metabolism. Since one of these effects is dependent on integrity of renal function, it is not surprising that disturbance of renal

3. Goadby, H. K., and Stacey, R. S.: (a) *Biochem. J.* **28**:2092, 1934; (b) **30**: 269, 1936.

4. (a) Ellsworth, R.: *J. Clin. Investigation* **11**:1011, 1932. (b) Ellsworth, R., and Futcher, P. H.: *Bull. Johns Hopkins Hosp.* **57**:91, 1935. (c) Ellsworth, R., and Howard, J. E.: *ibid.* **55**:296, 1934.

5. Thomson, D. L., and Pugsley, L. I.: *Am. J. Physiol.* **102**:350, 1932.

6. Selye, H.: *Endocrinology* **16**:547, 1932.

7. Thomson, D. L., and Collip, J. B.: *Physiol. Rev.* **12**:309, 1932; *Internat. Clin.* **4**:102, 1933.

8. McJunkin, F. A.; Tweedy, W. R., and McNamara, E. W.: *Am. J. Path.* **13**:325, 1937.

function should be reflected in disturbance of the function and effects of the parathyroid glands.

On injection of parathyroid extract and in many cases of clinical hyperparathyroidism marked diuresis is a prominent effect. While this is usually associated with marked increase of calcium and phosphorus in the urine, it appears to be independent of these factors. Shelling, Kajdi and Guth⁹ showed that the cause of death in acute overdosage with parathyroid extract was dehydration and loss of electrolytes as a result of the diuresis. Tweedy, Templeton and McJunkin¹⁰ showed that many of the described effects on tissues of acute parathyroid overdosage are not found in bilaterally nephrectomized animals.

Hyperparathyroidism causes a reversal of the usual path of excretion of calcium from the body. In the normal person most excreted calcium is found in the feces and but a small amount in the urine. In the patient with hyperparathyroidism most of the calcium is excreted by way of the kidneys; in addition, the total of calcium excreted is increased.

These rather complex and not fully understood interrelationships between parathyroid function, kidney function and the metabolism of calcium and phosphorus give some help in clarifying relationships between hyperparathyroidism and renal disease, as will be discussed in succeeding sections.

EFFECT OF PRIMARY DECREASE OF RENAL FUNCTION ON THE PARATHYROIDS

A considerable mass of evidence has now accumulated indicating that renal insufficiency may give rise to parathyroid hyperplasia and hyperfunction. These observations are of considerable value in elucidating one phase of the parathyroid-kidney relationship; they suggest a mechanism of stimulation and function of the parathyroid glands.

Experimental Observations.—Significant experimental demonstration of the effect of renal damage on the parathyroids has been given by Pappenheimer and his associates.¹¹ They produced renal damage in rats by unilateral nephrectomy and partial destruction of the remaining kidney by thermocautery. This reduction in functional renal tissue led to a decided increase in the size of the parathyroids. The use of the thermocautery produced a nephritic lesion in the remaining kidney tissue, and the severity of these nephritic changes (and so, presumably, the

9. Shelling, D. H.; Kajdi, L., and Guth, L.: *Endocrinology* **22**:225, 1938.

10. Tweedy, W. R.; Templeton, R. D., and McJunkin, F. A.: (a) *Am. J. Physiol.* **115**:514, 1936; (b) *Endocrinology* **21**:55, 1937.

11. (a) Pappenheimer, A. M.: *J. Exper. Med.* **64**:965, 1936. (b) Pappenheimer, A. M., and Wilens, S. L.: *Am. J. Path.* **11**:73, 1935.

degree of deficiency of renal function) correlated with the degree of parathyroid enlargement. Bony lesions comparable to osteitis fibrosa cystica developed. The animals with decreased renal function were more sensitive to the injection of parathyroid extract, as measured by its effect on bone. That the parathyroid hyperfunction so induced may also, in turn, have its effect on the kidney has been revealed by the experiments of Donahue, Spingarn and Pappenheimer,¹² who found that the calcium content of the residual renal tissue was increased in proportion to the parathyroid enlargement. Removal of the parathyroids prevented this increase in renal calcium.

Pollack and Siegal¹³ refer to a study by Hone on 'a syndrome occurring in dogs with renal insufficiency, characterized by osteoporosis of the skull, hyperplasia of the parathyroids and calcium deposits in soft tissues. They expressed the belief that this was a comparable disorder occurring in dogs.

Clinical Observations.—Virchow¹⁴ noted metastatic calcification in cases of nephritis, and since that time the association of renal lesions and deposition of calcium has been frequently noted. Only recently, however, have the parathyroids been carefully examined in renal disease, and the reasons for the previously noted changes have become clearer. Bergstrand¹⁵ noted in routine autopsies the frequency with which parathyroid enlargement accompanies renal damage and presented a study of 10 cases of renal disease associated with enlargement of the parathyroid glands. Vines¹⁶ remarked on the occurrence of parathyroid hyperplasia in patients with chronic nephritis but did not give details. Box and de Wesselow¹⁷ studied a patient with chronic nephritis whose blood calcium varied up to 20.1 mg. in 100 cc. and from clinical evidence suggested hyperparathyroidism. Lack of anatomic studies makes it difficult to determine the primary factor in this case.

Both Koopman¹⁸ and Radnai¹⁹ made histologic studies of the parathyroids in cases of chronic nephritis and failed to determine changes which they regarded as significant, though Radnai noted increased numbers of oxyphilic cells. Weights and measurements of the glands are not recorded. More recently, extensive detailed studies of the para-

12. Donahue, W.; Spingarn, C., and Pappenheimer, A. M.: J. Exper. Med. 66:697, 1937.

13. Pollack, H., and Siegal, S.: J. Mt. Sinai Hosp. 2:270, 1936.

14. Virchow, R.: Virchows Arch. f. path. Anat. 8:103, 1855.

15. Bergstrand, H.: Acta med. Scandinav. 53:644, 1921; 54:539, 1921.

16. Vines, H. W. C.: The Parathyroids in Relation to Disease, London, Edward Arnold & Co., 1924.

17. Box, C. R., and de Wesselow, O. L. V.: Lancet 2:543, 1925.

18. Koopman, H.: Frankfurt. Ztschr. f. Path. 25:342, 1921.

19. Radnai, P.: Frankfurt. Ztschr. f. Path. 46:97, 1933.

thyroids of patients with renal diseases have been made by Pappenheimer and Wilens,^{11b} Castleman and Mallory²⁰ and Gilmour and Martin.²¹ Also there have appeared a number of reports of single cases of renal disease in which parathyroid hyperplasia and effects of excessive function were outstanding.

Pappenheimer and Wilens^{11b} published studies of 21 cases of chronic renal disease, using as controls 72 miscellaneous cases of other than renal conditions. The renal lesions in the 21 cases were of various types and accompanied by varied degrees of disturbance of renal function. The observers found an increase in the weight of the parathyroids roughly proportional to the severity and extent of the renal lesions and to the degree of clinical insufficiency. With severe lesions the increase in weight was over 100 per cent. The enlarged glands had a more compact cellular structure with a smaller proportion of adipose tissue, and the large water-clear type of cell appeared dominant.

Castleman and Mallory^{20b} studied the parathyroids in 12 cases of chronic glomerular nephritis. In all but 2 of the cases there was gross enlargement of one or more glands. Microscopically, however, they found evidence of hyperplasia in all glands in every case. The fact that in some cases glands that were within normal limits of size showed satisfactory criteria of hyperplasia suggests that weights and measurements of parathyroids without skilled microscopic examination do not exclude the presence of hyperparathyroidism in a case of renal disease. It may be suggested, awaiting further study for proof or disproof, that in all cases of renal disease in which there is deficiency of function there is also some degree of overactivity of the parathyroid glands.

The same authors also describe 15 cases of assorted renal diseases other than glomerular nephritis in which there were mild degrees of parathyroid hyperplasia, although in 6 cases the glands showed no increase in size.

Gilmour and Martin²¹ made extensive statistical studies of the weight of the parathyroid glands. They found definitely higher mean weights in cases of nephritis and other renal diseases than in other disease groups. They also noted that in the cases of renal disease the glands had a higher percentage of parenchyma per unit of weight. In 7 of 32 cases of the nephritis reported on, the weights of the parathyroid parenchyma were above the upper limit of normal which they had established. In a case of very long-standing chronic nephritis associated with widespread calcification in soft tissues and generalized osteitis fibrosa, the parathyroid tissue weighed 2,569 mg. In the first case they described, one of early

20. Castleman, B., and Mallory, T. B.: (a) *Am. J. Path.* **11**:1, 1935; (b) **13**:553, 1937.

21. Gilmour, J. R., and Martin, W. J.: *J. Path. & Bact.* **44**:431, 1937.

nephritis, there was great enlargement and hyperplasia of only one gland, while the other glands were normal in size and structure. It appears unlikely that the parathyroid change was secondary in this case. They also mentioned a case of nephritis in a late stage in which only three glands were found, but with weights exceeding the normal limit. In a group of 25 cases of renal disease other than nephritis they found 3 cases in which the weight of the parathyroid parenchyma exceeded normal. Two of these were cases of chronic pyelonephritis, and the third was a case of renal rickets.

Individual cases of hyperparathyroidism apparently initiated by renal insufficiency have been reported by Hubbard and Wentworth,²² Barr and Bulger,²³ Albright,^{2a, b} Kluge,²⁴ Pollack and Siegal,¹³ Nelson²⁵ and Pons and Pappenheimer.²⁶ There is also the Cabot case.²⁷ Excluded from this number are cases of renal dwarfism and cases in which the parathyroid changes were limited to a single gland. Pons and Pappenheimer²⁶ in their tables gave some information regarding 2 other unpublished cases. Detailed descriptions of all these cases are easily available and will not be repeated here. The diversity of the actual lesions in the kidneys is noteworthy, however. Suppurative nephritis, subacute and chronic nephritis, arteriolosclerotic atrophy, polycystic disease and hydronephrosis have all been found to lead to parathyroid enlargement. In those cases in which the hyperparathyroidism was prominent clinically and easily diagnosed by the usual clinical criteria for hyperparathyroidism, the renal insufficiency was severe and had lasted over a long period with very slow progression. In some cases the classic lesions of osteitis fibrosa cystica resulted.

Fowweather and Pyrah²⁸ studied the blood calcium and inorganic phosphorus in 102 cases of various types of renal disease, and in about 50 per cent found the blood calcium to be over 11 mg. in 100 cc. Their results showed a tendency toward increase of blood calcium in widely different types of disease involving the kidneys. The changes in blood calcium and phosphorus generally associated with renal disease are a decrease in calcium and an increase in phosphorus. These changes, however, are not usually demonstrable until the renal disease is con-

22. Hubbard, R. S., and Wentworth, J. A.: *Proc. Soc. Exper. Biol. & Med.* **18**:307, 1920-1921.

23. Barr, D. P., and Bulger, H. A.: *Am. J. M. Sc.* **179**:449, 1930.

24. Kluge, E.: *Virchows Arch. f. path. Anat.* **298**:406, 1936.

25. Nelson, A. A.: *Arch. Path.* **24**:30, 1937.

26. Pons, J. A., and Pappenheimer, A. M.: *Puerto Rico J. Pub. Health & Trop. Med.* **13**:115, 1937.

27. Chronic Glomerular Nephritis; Secondary Parathyroid Hyperplasia, Cabot Case 22072, *New England J. Med.* **214**:320, 1936.

28. Fowweather, F. S., and Pyrah, L. N.: *Proc. Roy. Soc. Med.* **31**:593, 1938.

siderably advanced, and from the aforementioned results it may be that there is some stimulus in early renal disease which causes parathyroid hyperactivity, which in some cases more than counterbalances the tendency toward high phosphorus and low calcium.

Renal Dwarfism.—It is not intended to review here the subject of renal dwarfism (renal rickets). Various aspects have been reviewed by Mitchell and his collaborators,²⁹ Maddox,³⁰ Ellis and Evans,³¹ Park and Eliot³² and Price and Davie.³³ However, it has recently become evident that in many cases, and probably in all, the parathyroid glands play an essential role in the pathogenesis of the condition.

Although this condition is said to have been first described by Stenier and Neurenter³⁴ in 1870, it was not a clearly defined condition until the studies of Fletcher³⁵ appeared in 1911; it was further elucidated by Miller and Parsons,³⁶ Naish,³⁷ Barber,³⁸ Greene³⁹ and Parsons.⁴⁰

Duken⁴¹ suggested that there was an endocrine factor in renal rickets and pointed to the parathyroids as possibly involved. The involvement of the parathyroid glands in characteristic renal dwarfism was actually noted by Langmead and Orr⁴² and by Smyth and Goldman.⁴³ Shelling and Remson,⁴⁴ Gilmour and Martin,²¹ Price and Davie³³ and Howard⁴⁵ reported further instances in which studies of the parathyroid glands were made.

In the case reported by Langmead and Orr⁴² death occurred at the age of 20, and four large parathyroids were found. The skeletal changes were consistent with hyperparathyroidism.

29. (a) Mitchell, A. G.: *Am. J. Dis. Child.* **40**:101 and 345, 1930. (b) Mitchell, A. G., and Guest, G. M.: *Ohio State M. J.* **27**:134, 1931; *J. Pediat.* **3**:192, 1933.

30. Maddox, K.: *M. J. Australia* **1**:487, 1932.

31. Ellis, A., and Evans, H.: *Quart. J. Med.* **2**:231, 1933.

32. Park, E. A., and Eliot, M. M.: *Renal Hyperparathyroidism with Osteoporosis (Osteitis) Fibrosa Cystica*, in Brennemann, J.: *Practice of Pediatrics*, Hagerstown, Md., W. F. Prior Company, Inc., 1937, vol. 3, chap. 29.

33. Price, N. L., and Davie, T. B.: *Brit. J. Surg.* **24**:548, 1937.

34. Stenier and Neurenter, cited by Howard.

35. Fletcher, H. M.: *Proc. Roy. Soc. Med.* **4**:95, 1911.

36. Miller, R., and Parsons, L. G.: *Brit. J. Child. Dis.* **9**:289, 1912.

37. Naish, A. E.: *Brit. J. Child. Dis.* **9**:337, 1912.

38. Barber, H.: (a) *Lancet* **1**:18, 1920; (b) *Guy's Hosp. Rep.* **71**:62, 1922; (c) **76**:307, 1926.

39. Greene, C. H.: *Am. J. Dis. Child.* **24**:1, 1922.

40. Parsons, L. G.: *Arch. Dis. Childhood* **2**:1 and 198, 1927.

41. Duken, J.: *Ztschr. f. Kinderh.* **46**:137, 1928.

42. Langmead, F. S., and Orr, J. W.: *Arch. Dis. Childhood* **8**:265, 1933.

43. Smyth, F. S., and Goldman, L.: *Am. J. Dis. Child.* **48**:596, 1934.

44. Shelling, D. H., and Remson, D.: *Bull. Johns Hopkins Hosp.* **57**:158, 1935.

45. Howard, T. L.: *Am. J. Surg.* **40**:323, 1938.

The case reported by Smyth and Goldman⁴³ was that of a boy aged 14, with a history of glomerular nephritis, marked impairment of renal function without hypertension, and generalized demineralization of bone. Autopsy showed five enlarged, diffusely hyperplastic parathyroid glands, composed mainly of chief cells with moderate numbers of oxyphilic cells. The urinary tract showed a hypertrophied, trabeculated bladder, bilateral hydroureter and hydronephrosis. Calcium deposition in kidney tissue was prominent, and calcium deposition was present in numerous other organs and tissues.

The report by Shelling and Remson⁴⁴ has certain distinctive features, in that an increased amount of parathyroid hormone was demonstrated in the blood prior to death by the hypercalcemia test of Hamilton and Schwartz.⁴⁶

Price and Davie³³ reported a case:

A boy of 14 had suffered from thirst and polyuria since the age of 3 years. Deformities of the feet and legs were noted at 8 years and were progressive. Roentgenograms of the whole skeleton showed the characteristic changes of renal rickets. The urine was of low specific gravity, and the kidneys had no ability to concentrate it. Blood urea was 318 mg., serum calcium 13.6 mg. and serum phosphates 6.5 mg. in 100 cc. The boy appeared in comparatively good health, considering the degree of renal inadequacy, but there was rapid termination with uremia. Autopsy showed four enlarged parathyroid glands, measuring 10 by 6 by 4 mm., 15 by 6 by 4 mm., 15 by 8 by 6 mm. and 11 by 10 by 5 mm. (average normal size about 5 by 3 by 2 mm.). The changes found in the bones were essentially those of hyperparathyroidism. Microscopically, the parathyroid hyperplasia was uniform throughout and composed mainly of chief cells, but with a few oxyphils. The findings in the single kidney which was removed are particularly interesting. The kidney was very small and pale, with a diffusely granular surface and an adherent capsule. There was no evidence of pelvic dilatation or pyelitis. Microscopically there were seen many obliterated and partially obliterated glomeruli, but no evidence of active glomerular inflammation. Many tubules were greatly dilated, and some formed cysts. The main reaction was interstitial. The interstitial tissue was greatly increased in amount and showed infiltration with chronic inflammatory cells. There were numerous small foci of interstitial calcification, particularly within or immediately adjacent to basement membranes of tubules. No glomerular or intratubular calcification was noted. Just beneath the tubular basement membranes were small bodies which, the authors suggest, may have been calcium soaps. The vascular changes in the kidney were very slight.

Howard⁴⁵ likewise reported a case:

A 16 year old boy had shown deficient growth from birth. Bony deformities developed before school age. There was moderate frequency of urination with polyuria. The specific gravity of the urine was very low and fixed. The blood nonprotein nitrogen was 120 mg., the calcium 9.4 mg. and the phosphorus 11.3 mg. The nonprotein nitrogen in the blood rose to 300 mg. shortly before death. There was no hypertension. At autopsy three greatly enlarged parathyroid glands were

⁴⁶ Hamilton, B., and Schwartz, C.: *J. Pharmacol. & Exper. Therap.* **46**: 285, 1932.

located, the largest measuring 15 mm. in maximal diameter. Microscopically, the glands all showed dense hyperplasia of chief cells. The skeletal changes were those of osteitis fibrosa. The kidneys were very small and microscopically were characterized by marked interstitial changes, with calcium deposits in and beneath the basement membranes of tubules, and by tubular dilatation, but showed no evidence of active glomerulitis or more than slight vascular changes.

A number of other cases presented a picture so closely resembling renal dwarfism and hyperparathyroidism that they have been reported by one observer as instances of the former condition and by another observer as instances of the latter. Thus the case reported by Davies-Colley⁴⁷ is reported by Bulger and his co-workers⁴⁸ as an instance of hyperparathyroidism and by Mitchell^{29a} as an example of renal rickets, and Hutinel's⁴⁹ case, often cited as one of renal rickets, is mentioned by Shelling⁵⁰ as one of hyperparathyroidism. The cases of Cockayne and Lander⁵¹ and Lightwood⁵² are probably also examples of this condition.

An accurate evaluation of all the factors in the pathogenesis of renal dwarfism, with which all students of the subject are in agreement, has not yet been made, and for such an evaluation more data are required. Undoubtedly in many cases the initiating factor in the disease is a renal lesion. This renal lesion is often a congenital disturbance or obstruction, but various types of lesions have been noted. The essential character of the renal disease, however, is that it must develop before endochondral bone growth is completed and give rise to a severe degree of renal insufficiency which continues over a long period. The progressive nature of the usual type of nephritis does not allow it to meet these necessary conditions except in unusual cases, and this probably accounts for the comparative rarity of renal dwarfism as a complication of the nephritides of childhood.

From the facts that both clinical and experimental renal insufficiency produces parathyroid hyperplasia and hyperfunction and that parathyroid hyperplasia and hyperfunction have been demonstrated in a small number of cases of renal dwarfism, the evidence that the parathyroids play an essential role in this condition is very convincing. The lesions found in the bones are entirely comparable to those of osteitis fibrosa cystica, of renal hyperparathyroidism in adults and of experimental osteitis fibrosa produced by injections of parathyroid extract (Bodansky, Blair

47. Davies-Colley, J. N. C.: *Brit. M. J.* **1**:667, 1884.

48. Bulger, H. A.; Dixon, H. H.; Barr, D. P., and Schregardus, O.: *J. Clin. Investigation* **9**:143, 1930.

49. Hutinel, V.: *Gaz. d. hôp.* **80**:27, 1912.

50. Shelling, D. H.: *The Parathyroids in Health and in Disease*, St. Louis, C. V. Mosby Company, 1935.

51. Cockayne, E. A., and Lander, F. P. L.: *Arch. Dis. Childhood* **7**:321, 1932.

52. Lightwood, R.: *Arch. Dis. Childhood* **7**:193, 1932.

and Jaffe;⁵³ Bodansky and Jaffe⁵⁴), though modified by an effect on bones undergoing endochondral ossification, which gives rise to the stunting of longitudinal growth. This sequence in the development of renal dwarfism is backed by the experimental work of Pappenheimer,^{11a} who produced a comparable condition in young rats by producing renal insufficiency and keeping them on a deficient calcium intake. If the diet contained sufficient calcium and phosphorus, the parathyroid hyperplasia was only exceptionally accompanied by osteofibrotic skeletal changes. On a very low calcium intake the skeletal lesions were similar to those of marked rickets, whereas with a moderately deficient calcium intake the skeletal lesion was osteitis fibrosa. Thompson⁵⁵ prepared an extract of the parathyroid glands, which he noted would retard and limit growth in rats. Jaffe and Bodansky⁵⁶ noted that dogs treated by injections of parathyroid extract for long periods were stunted. Shelling, Asher and Jackson⁵⁷ reported moderate retardation of growth of rats receiving daily injections of parathyroid extract. It thus appears that renal dwarfism is but a modification of the renal hyperparathyroidism that occurs in adults, a modification in which the disease is of such severity and chronicity as to produce skeletal lesions. With this conclusion Park and Eliot³² are in complete agreement, and in their excellent review they have convincingly outlined the pathogenesis of this condition. The evidence for other factors in many of the cases cannot, however, be overlooked, and the presence of infantilism and other endocrine disturbances in some cases is not so easily explained on this basis. Several authors (Chown;⁵⁸ Roberts;⁵⁹ Chown and Lee⁶⁰) have suggested that the pituitary gland may be involved. Evidence that this gland may play a part in stimulation of the parathyroids has been produced by Houssay,⁶⁰ Hoffman and Anselmino,⁶¹ Hertz and Krane⁶² and others.

53. Bodansky, A.; Blair, J. E., and Jaffe, H. L.: *J. Biol. Chem.* **88**:629, 1930.

54. Bodansky, A., and Jaffe, H. L.: *J. Biol. Chem.* **93**:543, 1931; *J. Exper. Med.* **53**:591, 1931.

55. Thompson, J. H.: *J. Physiol.* **70**:xli, 1930.

56. Jaffe, H. L., and Bodansky, A.: *J. Exper. Med.* **52**:669, 1930.

57. Shelling, D. H.; Asher, D. E., and Jackson, D. A.: *Bull. Johns Hopkins Hosp.* **53**:348, 1933.

58. (a) Chown, B.: *Canad. M. A. J.* **35**:134, 1936; (b) *Brit. J. Surg.* **23**:552, 1936; (c) *Canad. M. A. J.* **37**:16, 1937. (d) Chown, B., and Lee, M.: *Am. J. Dis. Child.* **53**:117, 1937. (e) Chown, B.; Lee, M., and Teal, J.: *Canad. M. A. J.* **35**:513, 1936; **36**:7, 1937.

59. Roberts, J. F.: *Ann. Int. Med.* **9**:1729, 1936.

60. Houssay, B. A.: *Certain Relations Between Parathyroids, Hypophysis and Pancreas*, in *Harvey Lectures, 1934-1935*, Baltimore, Williams & Wilkins Company, 1936, p. 116.

61. Hoffman, F., and Anselmino, K. J.: *Klin. Wchnschr.* **13**:44, 1934.

62. Hertz, S., and Krane, A.: *Endocrinology* **18**:350, 1934.

Mitchell ^{20a} attributed the lesions in the skeleton to a deficiency in calcium absorbed from the intestine. He suggested that in the presence of renal insufficiency the intestinal mucosa may excrete waste endogenous phosphates, instead of the kidneys, the phosphorus forming with ingested calcium insoluble calcium phosphate, thus preventing absorption. Albright ^{2c} also did not agree that the parathyroids have a direct effect in the release of calcium from the bones and the production of skeletal lesions. The reasons he gave for believing that the hormone does not act on bone tissue are as follows. 1. The bones are not abnormal in hypoparathyroidism. 2. Primary hyperparathyroidism may exist without evidence of bone changes. 3. Bone changes in hyperparathyroidism can be made to regress by means of diet alone. On the other hand, convincing evidence has been produced indicating an effect of parathyroid extract directly on bones, even in the absence of the kidneys. Jaffe, ⁶³ Thomson and Collip, ⁷ Selye ⁶ and McJunkin and his associates ⁸ found a definite reaction in bony tissue following injection of parathyroid extract. While such details of the mechanism may be in dispute, the evidence that hyperparathyroidism is an essential part of renal dwarfism appears overwhelming. Park and Eliot ³² made the suggestion that in these cases the disease should be termed renal hyperparathyroidism with osteitis fibrosa cystica. This term defines the underlying condition, indicates the pathogenesis of the disease and describes the condition in the bones (albeit inaccurately). Its general adoption would relieve the confusion attending the term "renal rickets" and identify the condition with its counterpart in adults.

Both Shelling ⁵⁰ and Park and Eliot ³² emphasized that it may be very difficult in individual cases to distinguish renal hyperparathyroidism from primary or idiopathic hyperparathyroidism, particularly in the terminal stages, when the two may be identical. Shelling ⁵⁰ puts great emphasis on the history of renal symptoms antedating evidence of changes in the skeleton. This would not appear to be always reliable. In MacCallum's ¹ case the nephritis was primary as regards the time at which it became evident, yet the parathyroids showed a single adenoma rather than diffuse hyperplasia. Albright, Drake and Sulkowitch ²¹ pointed out that in many cases of primary hyperparathyroidism (with a parathyroid adenoma) renal symptoms may appear before bone symptoms or roentgen evidence of skeletal disease and that one should not wait for bone symptoms to make a diagnosis of hyperparathyroidism. They favored a high level of serum calcium as being of value in suggesting primary hyperparathyroidism as opposed to a disease in which renal insufficiency is the first step. The presence of calcium deposits in the kidney or of renal calculi is not, as Shelling ⁵⁰ suggested, a distinguishing

63. Jaffe, H. L.: *Arch. Path.* **16**:63 and 236, 1933.

characteristic of primary hyperparathyroidism alone, for these findings are not infrequent in cases of renal rickets. Again experimental work has been done which confirms these findings. Donahue, Spingarn and Pappenheimer¹² found increased calcium content in the remaining portions of damaged kidneys in rats in the presence of hyperplastic parathyroids. That this was due to parathyroid action was shown by the absence of this increase in renal calcium when the parathyroids were removed.

The actual lesions in the genitourinary tract in renal rickets divide themselves into two groups. In one group there are lesions of a congenital nature, either a cystic disorder of the kidneys or some abnormality of the lower part of the urinary tract resulting in dilatation of the ureters and hydronephrosis. In the other group are those peculiar changes of the kidney which commonly have been called chronic interstitial nephritis. Here there is advanced glomerular destruction and gross tubular dilatation, but evidences of antecedent glomerulonephritis, such as thickened and adherent tufts or crescents in Bowman's capsules, are absent, as are usually, also, vascular changes, hypertension and cardiac hypertrophy. Instead there are progressive inflammation and fibrosis in interstitial tissues, with deposition of small amounts of calcium. This group is entirely analogous to that produced as a result of primary chronic hyperparathyroidism.

As has been stated by Price and Davie,³³ these findings suggest the possibility that the syndrome of renal rickets may have its beginnings either in the endocrine system (parathyroids or hypophysis) or in the genitourinary system. In either case the parathyroids play an essential part, and the end picture may be clinically the same. Distinction as to the origin may then be possible only by (1) evidence of a primary parathyroid origin from the presence of a single parathyroid tumor or (2) evidence of a primary renal origin from the presence of a congenital abnormality of the kidneys or of the urinary tract. Such a theory of multiple origins of renal rickets does much to explain certain findings which were difficult to fit into the picture on the basis of a purely endocrine origin.

Comment.—The change in the glands in renal hyperparathyroidism appear to be true hyperplasia, affecting all the glands in the same fashion, though not necessarily producing the same degree of enlargement in each gland. The increase in size of the glands may be truly enormous; in one case a single gland weighed nearly 5 Gm. (normal about 60 mg.). The size is mainly accounted for by true hyperplasia. The individual cells are not increased in size and are usually reported to be mainly of the small chief cell type, densely packed together, with decrease in the proportion of fat cells to parenchyma. The details of these changes

have been carefully studied and clearly described by Castleman and Mallory.^{20b} The epithelial cells are arranged in wider columns or in solid masses of cells without discernible columnar arrangement. In more advanced cases there may be a tendency toward acinar arrangement. There are no mitoses. Oxyphile cells are more numerous. The microscopic appearance is diffuse and uniform throughout all the glands, though the glands may show variable degrees of increase in size. In the gross the glands are a creamy gray color, rather than light brown, and are firm in consistence.

That the parathyroid hyperplasia of renal disease is actually accompanied by increase in the amount of parathyroid hormone in the blood has been ably demonstrated by Highman and Hamilton,⁶⁴ using the hypercalcemia test in rabbits as devised by Hamilton and Schwartz⁴⁶ and Hamilton and Highman.⁶⁵ While Highman and Hamilton⁶⁴ did not find a strict proportionality between the rise of inorganic phosphorus in the blood and the increase in hormone, they suggested that the hyperphosphatemia was probably the stimulating factor.

The actual mechanism by which renal insufficiency brings about parathyroid hyperplasia will not be discussed in detail, as sufficient experimental work has not been performed to enable one to reach an unassailable conclusion. Parenteral administration of phosphates has produced parathyroid hyperplasia in rabbits (Drake and others⁶⁶), and several authors agree that retention of phosphates is the probable mechanism. Numerous experiments have indicated that the first effect of injection of parathyroid extract is to produce phosphate diuresis, and the hormone evidently has a direct effect on the kidney, lowering the threshold for phosphorus. Albright²¹ has recently suggested low blood calcium as the stimulus for parathyroid hyperplasia. Park and Eliot³² suggested that it is change in the calcium and inorganic phosphorous equilibrium of the blood which acts as a stimulus to parathyroid action. Acidosis may also be suggested as a possible stimulus to parathyroid hyperplasia. Solution of this problem awaits further investigation.

PRIMARY HYPERPARATHYROIDISM AND ITS EFFECT ON THE KIDNEYS

The Parathyroid Lesion.—Castleman and Mallory²⁰ and Albright and others^{2k, n} stressed the fact that two types of primary hyperparathyroidism (as far as is known) may occur, either of which may produce osteitis fibrosa and frequently renal disease. These types may be distinguished

64. Highman, W. J., Jr., and Hamilton, B.: J. Clin. Investigation **16**:103, 1937; Arch. Path. **26**:1029, 1938.

65. Hamilton, B., and Highman, W. J., Jr.: J. Clin. Investigation **15**:99, 1936.

66. Drake, T. G.; Albright, F., and Castleman, B.: J. Clin. Investigation **16**: 203, 1937.

by the morphologic aspects of the parathyroid glands, but the effects produced elsewhere (skeleton, kidneys) may be identical. The commoner type is characterized by an adenomatous enlargement of a single gland. In the other type there is a comparatively uniform increase in size of all four glands, as well as a distinctive microscopic appearance.

The adenomatous enlargement of a single gland has been described in detail by Castleman and Mallory^{20a} and by Hunter and Turnbull.⁶⁷ Such enlargement may be truly enormous. Sometimes it affects only part of a gland, leaving around it a rim of normal parathyroid tissue. In the majority of cases the growth is composed of types of chief cells; in a few it is composed of *wasserhelle* cells (Elsom, Wood and Ravdin⁶⁸); rarely, oxyphile adenoma, which apparently is hyperfunctioning, has been reported (Chown;^{58a,b,c} Venables;⁶⁹ Warren and Morgan⁷⁰).

Turnbull⁶⁷ expressed the opinion that the adenomatous enlargements were not autonomous new growths but enlargements due to functional hyperactivity. He compares this with the occurrence of localized areas of overactivity in the thyroid. Castleman and Mallory,²⁰ however, consider that these localized parathyroid enlargements represent neoplasia, basing this belief mainly on the localized character of the proliferative process.

The diffuse type of enlargement of the parathyroid glands in "primary" hyperparathyroidism is characterized by the presence of large *wasserhelle* cells making up the whole of the tissue, to the exclusion of other types of cells which are normally found in the glands. Albright and his associates^{2k, n} have made the most complete studies on this type of hyperparathyroidism. They believe this *wasserhelle* type of enlargement is secondary to some unknown stimulus. The glands may have a weight thirty to one hundred times normal. This increase in size can be explained mainly by hypertrophy of the cells, with little or no increase in number. The appearance is similar in all the glands. The cells are very large, with clear watery cytoplasm. There is increased tendency to glandular arrangement. Oxyphile cells, chief cells and mitoses are absent. Fat tissue is also absent. No case has been found in which the condition has been present in mild or transition forms, or in only a portion of a gland. Once the condition is present, it remains indefinitely, but there is little tendency to regeneration if a portion is removed at operation. The weight of the gland tissue and the degree of hyperparathyroidism appear to be definitely correlated.

67. Hunter, D., and Turnbull, H. M.: Brit. J. Surg. **19**:203, 1931.

68. Elsom, K. A.; Wood, F. C., and Ravdin, I. S.: Am. J. M. Sc. **191**:49, 1936.

69. Venables, J. F.: Guy's Hosp. Rep. **83**:194, 1933.

70. Warren, S., and Morgan, J. R. E.: Arch. Path. **20**:823, 1935.

The Renal Lesion.—Primary hyperparathyroidism associated with marked renal insufficiency has been reported by Elsom, Wood and Ravdin,⁶⁸ Baker and Howard,⁷¹ Bellin and Gershwin,⁷² Albright,²³ Anderson⁷³ and others.

Elsom, Wood and Ravdin⁶⁸ noted that the renal disease associated with primary hyperparathyroidism has certain features which distinguish it from the usual types of chronic nephritis. Hypertension, cardiac enlargement, edema and retinal exudates are usually absent or very inconspicuous. In many cases the subject appears to tolerate an unusual degree of kidney damage and insufficiency without much subjective discomfort. It seems possible to relate these distinctive clinical features to the morphologic peculiarities of the renal lesion. Vascular lesions are characteristically absent or slight, as is active glomerular involvement. Morphologically one would expect the greatest interference with tubular function, and this seems to be reflected in the inability to produce concentrated urine. Correction of the hyperparathyroidism might be expected to halt the progression of the lesion (Baker and Howard;⁷¹ Bellin and Gershwin⁷²), but improvement in renal function could occur only in early cases (Baker and Howard⁷¹).

The literature now contains many reports of cases of primary hyperparathyroidism. In a large proportion of the reported cases there was some evidence of disturbance of renal function or of the presence of renal calculi. Polyuria and polydipsia, associated usually but not necessarily with hypercalciuria, were among the earliest and most constant of symptoms. In later stages it was extremely common to find a slight amount of albumin and a few casts in the urine, with lowering and fixation of the specific gravity. Treatment in early stages sometimes produced improvement in renal function and ability to concentrate (Baker and Howard;⁷¹ Cooley;⁷⁴ Pemberton and Geddie;⁷⁵ Boyd, Milgram and Stearns,⁷⁶ and others). The presence of renal calculi, with associated obstruction and infection, may change the usual renal picture.

In some cases the evidence of renal disease overshadowed other evidences of hyperparathyroidism, and in recent years, since involvement of the kidneys has been more generally realized to be an occurrence in hyperparathyroidism, such cases have been reported more frequently.

71. Baker, B. M., Jr., and Howard, J. E.: Bull. Johns Hopkins Hosp. **59**: 251, 1936.

72. Bellin, D. E., and Gershwin, B. S.: Am. J. M. Sc. **190**:519, 1935.

73. Anderson, W. A. D.: Endocrinology **24**:122, 1939; J. Pediat., to be published.

74. Cooley, T. B.: Am. J. Dis. Child. **42**:691, 1931.

75. Pemberton, J. de J., and Geddie, K. B.: Ann. Surg. **92**:202, 1930.

76. Boyd, J. D.; Milgram, J. E., and Stearns, G.: J. A. M. A. **93**:684, 1929.

ANDERSON—HYPERPARATHYROIDISM

In the older literature on hyperparathyroidism renal changes were described occasionally, but careful histologic studies of the kidneys in cases of hyperparathyroidism rarely have been made.

Calcium precipitations in the form of renal calculi or parenchymal deposits or both occur with great regularity. Randall's⁷⁷ studies indicate that a parenchymal calcium deposit is the primary lesion in the cases in which renal calculi are found. Askanazy⁷⁸ in his case noted contracted kidneys with interstitial inflammation and many areas of calcification and interstitial inflammation. Molineus⁷⁹ described contracted kidneys in 2 cases. Harbitz⁸⁰ found interstitial inflammation and calcareous concretions of phosphates and carbonates. Marked calcium deposits were found by Hartwich,⁸¹ Parreira and Castro-Freire,⁸² Wanke,⁸³ Ask-Upmark,⁸⁴ Noble,⁸⁵ Peneche,⁸⁶ Hoffheinz⁸⁷ and MacCallum.¹

Knowledge of renal disease in association with hyperparathyroidism was summarized in 1934 by Albright and co-workers.²¹ They described the renal lesions as being of three types. Stone formation, which they found to be the most commonly mentioned renal complication, designated as type I. However, as stated in a foregoing paragraph, deposition of calcium in the parenchyma is probably primary, and so must be even more frequent than calculus formation, though less apt to be noticed, unless the deposit is so severe as to show in a roentgenogram or to be striking at postmortem examination.

This parenchymal calcium deposition, which they termed nephrocalcinosis, they designated as type II, stressing the occurrence of calcium deposits in the renal tubules and the presence in the urine of granular, calcium-containing casts. The extratubular mineral deposits, which are even more striking and important, are neglected by them. They suggested that the deposition of calcium in the kidney causes permanent and serious damage of the kidney, with chronic inflammatory changes and subsequent

77. (a) Randall, A.: *Surg., Gynec. & Obst.* **64**:201, 1937; (b) *Ann. Surg.* **105**:1009, 1937. (c) Randall, A.; Eiman, J. E., and Leberman, P. R.: *J. A. M. A.* **109**:1698, 1937.

78. Askanazy, M.: *Arb. a. d. Geb. d. path. Anat. Inst. zu Tübingen* **4**:398, 1904.

79. Molineus: *Arch. f. klin. Chir.* **101**:333, 1913.

80. Harbitz, F. J.: *J. M. Research* **32**:361, 1915.

81. Hartwich, A.: *Virchows Arch. f. path. Anat.* **236**:61, 1922.

82. Parreira, H., and Castro-Freire, L.: *Compt. rend. Soc. de biol.* **95**:1590, 1926.

83. Wanke, R.: *Beitr. z. klin. Chir.* **136**:664, 1926.

84. Ask-Upmark, E.: *Acta med. Scandinav.* **74**:284, 1930; *Acta chir. Scandinav.* **68**:551, 1931.

85. Noble, T. P.: *J. Bone & Joint Surg.* **14**:181, 1932.

86. Peneche, R.: *Zentralbl. f. allg. Path. u. path. Anat.* **37**:535, 1926.

87. Hoffheinz, S.: *Virchows Arch. f. path. Anat.* **256**:705, 1925.

sclerosis and contraction, the end point simulating chronic nephritis. With this conclusion other pathologists agree (Chown^{88a}) and disagree (Pappenheimer and Wilens^{11b}).

In type III, which they call "parathyroid poisoning," deposits of calcium occur in the kidney but, in addition, in various other organs, such as the lung, stomach and heart.

It is evident that in these three types the primary or essential factor as far as the kidneys are concerned is the deposition of calcium in the renal parenchyma, and thus division into types is only a clinical convenience. From the pathologic standpoint the important problem seems to be whether or not deposition of calcium per se damages kidney tissue to such an extent that renal function may be decreased.

It seems significant that almost constantly in cases of hyperparathyroidism in which histologic studies were carefully made a particular type of lesion has been described. This renal change is predominantly interstitial. There is interstitial infiltration by chronic inflammatory cells, interstitial fibrosis and interstitial deposition of calcium, particularly in or adjacent to tubular basement membranes. Tubular and intratubular calcium deposits, while usually present, are less prominent, particularly in late stages, and seem to be more characteristic of an acute phase. Frequently tubules are dilated and lined by flattened epithelium. Often small cysts are formed. Glomeruli are partially or completely fibrosed, but evidence of proliferative or exudative changes in glomeruli, such as are seen in glomerular nephritis, are absent. Vascular involvement is usually slight; when present, it appears to be an unrelated change.

Many of the calcium deposits, both those within tubules, associated with desquamation and destruction of tubular epithelium, and peritubular masses, may give rise to obstruction of the tubule and an effect on other portions of the nephron. The tubular dilatations and many of the obliterations of glomerular tufts are expressions of these obstructive effects. Other conditions in which tubular obstruction is productive of renal damage have been described by Forbus and his associates⁸⁸ in Bence Jones proteinuria and by Duguid^{89b} in lesions produced by calciferol and described in more detail elsewhere. It is obvious that widespread obstruction of tubules may severely damage the kidney and produce insufficiency. MacNider⁹⁰ described regeneration of tubular epithelial cells in a more resistant form after injury by uranium nitrate and mercury bichloride. No studies have been made which indicate whether or not such regeneration of tubular cells, with acquired resis-

88. Forbus, W. D.; Perlzweig, W. A.; Parfentjev, I. A., and Burwell, J. C., Jr.: *Bull. Johns Hopkins Hosp.* **57**:47, 1935.

89. Duguid, J. B.: (a) *Lancet* **2**:983, 1930; (b) **2**:987, 1938.

90. MacNider, W. de B.: *Ann. Int. Med.* **12**:147, 1938.

tance, may follow slighter degrees of damage by hyperparathyroidism. Such a process, however, may partially explain the immunity acquired by many experimental animals to injections of parathyroid extract.

Some of the interstitial calcium deposits may have been primarily in tubules and, following degeneration of the injured nephron, appear to be in interstitial tissue surrounded by more prominent fibrous tissue.

Parenchymal calculi may be formed by coalescence of calcium concretions or by ulceration of the tubular epithelium overlying a protruding peritubular mineral deposit, and subsequent precipitation from tubular urine. The development of calculi, with obstruction and infection, may modify the picture of the renal lesion.

Experimental Results.—Experimental work with parathyroid extract would naturally be looked to for the solution of the pathogenesis of the renal lesions in hyperparathyroidism. The results are somewhat disappointing. Most experimenters (Hueper;⁹¹ Learner;⁹² Cantarow, Stewart and Housel⁹³) produced acute hyperparathyroidism by large doses, a condition that does not simulate the relatively mild chronic hyperparathyroidism seen in man. Many animals seem to have or to develop tolerance toward the extract. Selye⁶ showed that in rats this apparent immunity which develops is really a change in the response of the tissues affected, in such a fashion that an osteoblastic rather than an osteoclastic action may be produced in bones. Whether or not there develops a change in the response of the kidney to continued administration of the parathyroid extract has not been demonstrated.

Hueper,⁹¹ Learner⁹² and Cantarow, Stewart and Housel⁹³ described the renal changes in dogs in experimental acute hyperparathyroidism. Their findings in the kidney are all essentially similar. Marked degenerative changes were found in the renal tubular epithelium. All portions of the cortical tubules were involved, and occasionally the collecting tubules to a lesser degree. Dilated tubules, some lined by flattened epithelium, were frequent. The amount of calcium deposited did not parallel the value of serum calcium. The mineral deposits were found in tubular epithelium and as casts in the tubular lumen. Hueper⁹¹ also described some calcification of tubular basement membranes and of Bowman's capsule.

McJunkin and his co-workers⁹⁴ and Cantarow and his associates⁹³ were emphatic that the calcification in experimental hyperparathyroidism is not metastatic in the sense that calcium is deposited in tissue otherwise

91. Hueper, W. C.: Arch. Path. **3**:14, 1927.

92. Learner, A.: J. Lab. & Clin. Med. **14**:921, 1929.

93. Cantarow, A.; Stewart, H. L., and Housel, E. L.: Endocrinology **22**:13, 1938.

94. McJunkin, F. A.; Tweedy, W. R., and Breuhaus, H. C.: Arch. Path. **14**: 649, 1932.

normal. Cantarow and his associates⁹³ invariably found regressive changes in the kidneys, which they believed contributed to the localization of the deposits. McJunkin and his associates⁹⁴ suggested that this injury was produced by disturbance of the calcium components of the tissue fluids of the cells themselves.

The most enlightening experimental studies are those of Chown, Lee and Teal.^{58e} They used young rats, giving them graduated parenteral doses of parathyroid extract over long periods. Early lesions consisted of intratubular calcium deposits, coarse and fine interstitial calcium deposits and extension of calcium masses into the tubular lumens, with a leukocytic reaction. Many of these deposits were only temporary. Late results (up to one hundred and seventy-four days) were very striking, with cystic tubular dilatation, occasionally irregular pelvic dilatation and chronic focal inflammatory reaction. Varying amounts of interstitial reaction and proliferation occurred, with some glomerular changes. Calcium deposits in glomeruli were not seen. Eventually, in late cases, calcium deposition was often small in amount and was present only in interstitial tissues. The resemblance of this to the findings in the kidneys in clinical cases of renal rickets and primary hyperparathyroidism is evident.

It seems from the evidence only fair to conclude that hyperparathyroidism may produce definite kidney disease. To decide whether this renal damage may be produced as a result of hypercalcemia per se or only with the addition of other factors is more difficult. Considerable evidence indicates that in hyperparathyroidism there are other assisting elements. Definite evidence of degenerative changes in the kidney in experimental acute hyperparathyroidism has been referred to in a foregoing paragraph. This is reflected in the common finding in patients with parathyroid adenoma of a loss of concentrating power by the kidneys, with a fixed low specific gravity of the urine, which may be relieved if the adenoma is surgically removed in early stages of the disease. That hypercalcemia alone may not be very injurious is indicated in the report by Reed⁹⁵ of a young adult human subject who maintained "hypercalcemia of 24 mg. for 8 days by vitamin D overdosage without evidence of toxicity or subsequent damage." Duguid^{89b} in experimental work with rats given acid sodium phosphate and calciferol found that what begins as a purely tubular degenerative lesion, with calcification, may eventually produce in the kidney microscopic changes very difficult to distinguish from an advanced stage of chronic glomerulonephritis. The primary change was a tubular degenerative change with focal calcification. Groups of degenerated convoluted tubules of the cortex either atrophied and left areas of fibrosis or became dilated and cystlike. The glomeruli in their turn degenerated, the capillaries becoming hyaline and

95. Reed, C. I.: J. A. M. A. **102**:1745, 1934.

their lumens obliterated, so that eventually the glomerular tufts were transformed into solid masses of hyaline substance, which later became fibrosed. These glomerular lesions were found only in the more severely affected animals which survived some months after the production of the initial lesion. The variety of histologic pictures produced often imitated the various forms of primary chronic nephritis in man.

Renal Calcification.—Because of the stress which has been put on the deposition of calcium in the kidney in the renal lesion of primary hyperparathyroidism, certain points about renal calcification will be reviewed. There is no doubt that in experimental acute hyperparathyroidism disturbance of renal function having no relation to deposition of calcium occurs, even though in such experiments extensive calcium masses may be laid down in the kidney in less than forty-eight hours. The anuria which regularly precedes death in acute hyperparathyroidism appears, however, to be related to loss of electrolytes, and the symptoms may be relieved by replacement of the deficient water and electrolytes. However, in the chronic forms of hyperparathyroidism the deposition of calcium appears to be a definite factor leading to damage of the kidney tissue and decreased efficiency of renal function, as well as to formation of calculi and resulting obstruction and infection.

The subject of pathologic calcification was reviewed by Barr.⁹⁶ He emphasized the rarity of calcium metastases in human diseases, and also the frequency with which they have been found related to nephritis. He stated that it is difficult to find a case of extensive metastatic calcification in which a renal element can be definitely excluded.

Factors of importance in the precipitation of calcium include the concentration of calcium, the concentration of phosphates and the degree of alkalinity of the serum and tissue fluids. Localized increase in alkalinity may cause deposition of calcium even without great increase in concentration of calcium and phosphates. If there is increase in concentration of calcium and (or) phosphates, as there may be in hyperparathyroidism, local relative alkalinity may still be a factor causing precipitation of calcium in a particular place. On this basis has been explained the frequency of calcium deposits in the lungs, stomach and kidneys. Kleinmann⁹⁷ emphasized that degenerating and devitalized tissues similarly have relative alkalinity.

Wells, Holmes and Henry⁹⁸ pointed out the peculiarity of the calcium deposits in the kidney when the tubules are the structures involved. Tubular calcification they compare to calcification of organic material

96. Barr, D. P.: *Physiol. Rev.* **12**:593, 1932.

97. Kleinmann, H.: *Virchows Arch. f. path. Anat.* **268**:686, 1928; *Biochem. Ztschr.* **196**:98, 146 and 161, 1928.

98. Wells, H. G.; Holmes, H. F., and Henry, G. R.: *Am. J. M. Research* **25**: 373, 1911-1912.

in the urine rather than to calcification of tissues with lime salts directly from the blood. The epithelium and tube cast deposits they found to contain calcium only as phosphate, whereas in interstitial tissues carbonate could be demonstrated as well, the calcium in the latter situation probably coming from the blood. In the urine calcium is present chiefly in the form of the acid phosphate. The common stain used to demonstrate the presence of calcium in tissues is silver nitrate. Wells and his co-workers⁹⁸ stated, and the statement has been fully confirmed by the work of Cameron,⁹⁹ that silver nitrate has no affinity for calcium and detects not calcium but certain acid radicals, particularly the phosphate.

Experimental and metabolic studies have indicated that increased calcium and phosphorus in the urine, as well as degenerative changes in kidney tissue, are characteristic features of hyperparathyroidism. Thus in acute phases of hyperparathyroidism, particularly, conditions are ripe for the deposition of calcium in renal tubules. The experiments of Chown^{58a,c,e} have shown that with progression of the renal lesion much of this tubular deposit is removed. Adequate studies of the amount of epithelial regeneration which may follow have not been made, but evidence indicates that even up to this stage there may be return of the kidney to more efficient function. In the later stages of the renal lesion, when chronic insufficiency of renal function is present, the deposition of calcium is predominantly interstitial. The extent of this interstitial accumulation of calcium is probably not realized because of the use of the common but inadequate silver nitrate method for the demonstration of calcium, which, as was emphasized in a foregoing paragraph, stains not the calcium but the phosphate radical. Extensive interstitial calcium deposits of this sort are a rare occurrence in any condition other than hyperparathyroidism. Experimentally induced hypervitaminosis D may produce a somewhat similar renal condition in an acute phase, as will be discussed separately. The extensive renal calcification sometimes accompanying high intestinal obstruction (Cooke;¹⁰⁰ Brown and co-workers¹⁰¹) is mainly tubular and dependent on dystrophic changes in tubular epithelium.

The part played by renal phosphatase in renal calcification has not been thoroughly investigated. Hyperparathyroidism with skeletal lesions is associated with increased serum phosphatase, but the phosphatase of the kidney tissue under these circumstances is not known. The work of Rosenheim and Robison¹⁰² suggested that renal phosphatase is a

99. Cameron, G. R.: J. Path. & Bact. **33**:929, 1930.

100. Cooke, A. M.: Quart. J. Med. **2**:539, 1933.

101. Brown, G. E.; Eusterman, G. B.; Hartman, H. R., and Rowntree, L. G.: Arch. Int. Med. **32**:425, 1923.

102. Rosenheim, A. H., and Robison, R.: Biochem. J. **28**:712, 1934.

factor of some importance for calcification in vitro. One might expect, though as yet without concrete evidence, that in hyperparathyroidism increase of phosphatase in kidney tissue would be an important factor causing the unusual type of renal calcification.

Renal Lithiasis.—The presence of renal calculi has been noted very frequently in cases of hyperparathyroidism. Among 75 cases in which parathyroid adenoma was proved to be present, collected by Compere,¹⁰³ there were 24 in which renal calculi were found. In the series of 32 cases of hyperparathyroidism reported by Hunter,¹⁰⁴ calculi were found in 10, or about the same proportion. Barney and Mintz¹⁰⁵ noted that of 65 cases of parathyroidism reported by various authors, stones were present in 15. In the series of cases reported by Barney and Mintz¹⁰⁶ from Boston almost 70 per cent showed renal stones, a much higher percentage than in the other collected instances that have been noted here. Albright and his associates²¹ mentioned calculous disease as the commonest renal complication of hyperparathyroidism in their cases.

It is evident then that renal formation of stones is one of the commonest results of hyperparathyroidism. Estimations as to the importance of the parathyroids in the general problem of the renal formation of stones have been variable. Barney and Mintz¹⁰⁵ stated that hyperparathyroidism was a factor in 10 per cent of cases of renal calculi, but later they¹⁰⁶ revised this figure to between 4 and 5 per cent. This figure was based on a series of 340 patients with urinary lithiasis, some of whom were proved by operation to be suffering from hyperthyroidism. In other clinics the proportion has been found to be much lower. Griffin, Osterberg and Braasch¹⁰⁷ found hyperparathyroidism to be a factor in less than 0.2 per cent of 1,206 cases of urinary lithiasis. Geographic location, a known factor of importance in calculous disease, would cause slight variation in such figures but hardly enough to account for such a discrepancy. Marquardt¹⁰⁸ in a small series of cases did not find hyperparathyroidism to be a factor. Fowweather and Pyrah²⁸ also agreed that primary hyperparathyroidism is a factor in only a very small proportion of cases of renal calculi.

Renal calculi are not common in children but have been noted in many who had renal dwarfism (Davies-Colley⁴⁷; Barber^{38b}; Parsons⁴⁰; Mitchell²⁹) which, as has been indicated, is associated with hyperpara-

103. Compere, E. L.: Arch. Surg. **32**:232, 1936.

104. Hunter, D.: Lancet **1**:897 and 946, 1930; Quart. J. Med. **24**:393, 1931; Brit. M. J. **1**:982 and 929, 1937.

105. Barney, J. D., and Mintz, E. R.: J. A. M. A. **103**:741, 1934.

106. Barney, J. D., and Mintz, E. R.: J. Urol. **36**:159, 1936; Brit. J. Urol. **8**:36, 1936.

107. Griffin, M.; Osterberg, A. E., and Braasch, W. F.: J. A. M. A. **111**:683, 1938.

108. Marquardt, C. R.: Wisconsin M. J. **36**:177, 1937.

thyroidism and frequently with calcium deposits in the kidneys. The probable mechanism by which renal calculi are formed in hyperparathyroidism has been revealed in the studies made by Randall⁷⁷ and by Chown, Lee and Teal.^{58c} Randall showed that the basic lesion in renal stone formation is a parenchymal deposit of calcium in the kidney substance, particularly in the walls and intertubular spaces of the renal papilla. These plaquelike calcium masses may lose their epithelial covering and be subjected to constant contact with calycine urine, acting then as a nidus on which further deposition may occur and being held in place until a stone of some size is formed. The association of these conditions with evidence of tubular damage has also been emphasized by Randall.

From the earlier discussion of the morphologic changes in the kidney, it is seen how frequently hyperparathyroidism produces just such an initiating lesion for the formation of stone. Tubular damage and interstitial calcium deposits are marked features. While the deposition of calcium is usually most prominent in the cortex, there is also frequent and sometimes severe involvement of the papillae. Randall and co-workers^{77c} described production of the characteristic lesion in a papilla by administration of parathyroid extract to a dog.

The experimental work of Chown, Lee and Teal^{58c} showed how the small calculi within kidney tissue may be formed. They described extension of peritubular calcium masses into the lumens of tubules in rats given parathyroid extract. "The epithelium overlying calcium might be seen to protrude a little into the lumen. By degrees it advanced, the calcium close behind. The tubules dilated like a celomic cavity. The solid cord of calcium carried the tubular epithelium before it, as fetal gut carries peritoneum; behind it the epithelium formed a mesentery. The mesentery disappeared, leaving a small, solid, calcium-filled, epithelial tube or ball lying free in a larger tube." Anderson⁷³ described a similar process in a case of hyperparathyroidism with renal calculi in man.

There is general agreement that persons suffering from renal lithiasis deserve to have the possibility of hyperparathyroidism investigated. Barney and Mintz¹⁰⁶ emphasized that in patients with hyperparathyroidism and renal calculi who had been treated successfully by surgical operation stones did not recur.

Comment.—Certain other conditions occasionally produce calcium deposits in the kidney and may result in a renal lesion similar in this respect to that of hyperparathyroidism. Certain skeletal diseases, such as multiple myeloma, have produced renal calcium deposits. Experimental administration of excessive amounts of vitamin D has produced very similar renal lesions, but no well authenticated cases of such lesions following administration of this vitamin in man have been described.

The renal lesions in multiple myelomatosis have been studied by Geschickter and Copeland,¹⁰⁹ Perla and Hutner¹¹⁰ and Forbus and his associates.⁸⁸ Perla and Hutner stressed the presence of nephrosis and calcium deposits in the renal tubules. This localization is apparently dependent on the degenerative changes associated with increased concentration of calcium in the tubular urine. The obstructive features, as stressed by Forbus in his consideration of this lesion, have already been mentioned; in this lesion they seem dependent on protein casts rather than on calcium casts as in hyperparathyroidism.

There is some confusion regarding the changes which may be produced in the kidney by experimental hypervitaminosis D. Much of the early work was done with preparations which contained a large proportion of other sterols (e. g., toxisterol) more toxic than calciferol. As a result, it is uncertain whether many of the lesions described were due to vitamin D or to toxisterol. Considerable doubt may be felt about the cases of death in infancy due to hypervitaminosis D as reported by Putschar¹¹¹ and by Thatcher.¹¹² While the exact dose of vitamin D in these cases cannot be estimated accurately, it does not seem to have been of an order that has been found toxic with the recent pure forms of vitamin D. Calcium deposits such as those described in these cases have been shown by Anderson⁷³ to be not uncommon in infants, in association with a variety of conditions.

The renal lesions produced by hypervitaminosis D have been described by many authors, among whom may be mentioned: Spies and Glover¹¹³; Gough, Duguid and Davies¹¹⁴; Reed, Dillman, Thacker and Klein,¹¹⁵ and Duguid.^{89b} A degenerative lesion (nephrosis) and calcification both occur, but the two appear to be largely independent. The calcification frequently affected tubular basement membranes and glomerular capsules, as well as tubular epithelium. In late stages the renal lesions of experimental hypervitaminosis D and hyperparathyroidism may bear a striking resemblance. This suggests that either vitamin D exerts its effects on calcium metabolism by way of the parathyroid glands or the disturbance in calcium metabolism in the two conditions is the main factor in the production of the renal lesions. The question

109. Geschickter, C. F., and Copeland, M. M.: *Arch. Surg.* **16**:807, 1928.

110. Perla, D., and Hutner, L.: *Am. J. Path.* **6**:285, 1930.

111. Putschar, W.: *Ztschr. f. Kinderh.* **48**:269, 1929.

112. Thatcher, L.: *Edinburgh M. J.* **38**:457, 1931.

113. Spies, T. D., and Glover, E. C.: *Am. J. Path.* **6**:485, 1930.

114. Gough, J.; Duguid, J. B., and Davies, D. R.: *Brit. J. Exper. Path.* **14**: 137, 1933.

115. Reed, C. I.; Dillman, L. M.; Thacker, E. A., and Klein, R. I.: *J. Nutrition* **6**:371, 1933.

as to whether or not vitamin D produces its effects by way of the parathyroid glands has been thoroughly discussed recently by Best and Taylor ¹¹⁶ and by Shelling ⁵⁰ and will not be reviewed here except to say that the weight of evidence appears to be against this hypothesis. If, then, two conditions produce by independent methods similar disturbances in calcium metabolism and also produce similar renal lesions, it suggests that in hyperparathyroidism the disturbance in calcium metabolism may be the essential factor in the production of the kidney disease.

SUMMARY

The parathyroid glands and the kidneys have a close functional relationship. One of the first and most characteristic effects of the administration of parathyroid extract is an increase in renal secretion of phosphate. Deficiency of renal function stimulates hyperplasia and hyperfunction on the part of the parathyroid glands. The actual stimulating factor is not definitely known but is probably some disturbance of calcium or phosphorus balance resulting from the renal deficiency. Parathyroid hyperplasia and hyperfunction are probably present in some degree in all cases in which there is deficiency of renal function. If the disturbance is severe and long continued, a clinical picture characteristic of osteitis fibrosa cystica, in adults, or renal rickets, in children, is produced. These conclusions have received both clinical and experimental confirmation.

Hyperparathyroidism may produce renal lesions of a distinctive type and result in renal failure. Such hyperparathyroidism may be due to a localized adenomatous overgrowth of a single parathyroid or to a peculiar diffuse hypertrophy of all the parathyroids. The resulting disturbance in calcium metabolism appears to be the main cause of the damage in the kidney, though other factors may also have some part in the development of the renal disease. Calcium deposits in the kidney are the characteristic feature. In acute hyperparathyroidism the calcium may be mainly intratubular, but in chronic hyperparathyroidism it is interstitial and peritubular and is accompanied by interstitial fibrosis and cellular infiltration. Damage to the kidney appears to be mainly the result of tubular obstruction. Renal calculus formation is very frequent and develops on the basis of parenchymal calcium concretion. Hyperparathyroidism is, however, the underlying cause of only a very small proportion of renal calculi.

116. Best, C. H., and Taylor, N. B.: *The Physiological Basis of Medical Practice: A University of Toronto Text in Applied Physiology*, Baltimore, William Wood & Company, 1937, p. 1111.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, Etc.—Charles C. Okell, formerly professor of bacteriology at the University College Hospital Medical School, London, died on February 9 at the age of 50.

Georg Strassmann, formerly extraordinary professor of forensic and social medicine in the University of Breslau (Germany), has become a member of the department of forensic medicine in New York University.

The Rockefeller Foundation has made a grant of \$2,000 for the continuation in 1939 of the studies on malaria by William H. Taliaferro at the University of Chicago.

According to *Science*, the Commonwealth Fund has made a grant of \$8,360 to the Institute of Pathology at Western Reserve University to support for a year studies on the chemistry of immunity under E. E. Ecker. Progress may lead to similar grants for two more years.

Society News.—The annual meeting of the American Society of Clinical Pathologists will be held in St. Louis, May 12, 13 and 14. The seminar will be held on Sunday, May 14. The Hotel Desota will be the headquarters.

The twenty-fourth annual meeting of the American Association of Industrial Physicians and Surgeons with the American Conference on Occupational Diseases and Industrial Hygiene will be held at the Hotel Statler, Cleveland, June 5, 6, 7 and 8, 1939. Information regarding hotel accommodations, etc., may be obtained from A. G. Park, 540 North Michigan Avenue, Chicago.

The third International Congress for Microbiology will be held in the Waldorf-Astoria Hotel, New York, Sept. 2-9, 1939. The general secretary is Dr. Martin H. Dawson, 620 West 168th Street, New York City.

Abstracts from Current Literature

TO SAVE SPACE THE ORIGINAL TITLES OF ABSTRACTED ARTICLES SOMETIMES
ARE SHORTENED

Experimental Pathology and Pathologic Physiology

CONGENITAL UNIVERSAL INSENSITIVENESS TO PAIN. F. R. FORD and L. WILKINS,
Bull. Johns Hopkins Hosp. 62:448, 1938.

Three cases are reported in which there occurred in children between the ages of 7 and 8 years a congenital indifference to potentially painful stimuli, leading on one occasion to severe burns, multiple fractures and other serious injuries. Except for the disregard of pain, the authors were able to demonstrate no other evidence of disease or defect of the nervous system. They give various reasons for their belief that these children do not have true analgesia but present a defective reaction to the crude sensation of pain which makes that sensation a matter to be disregarded. A small number of cases of a similar nature recorded in medical literature are mentioned. The authors are inclined to believe that such a condition represents a congenitally defective development in the sensory system which involves selectively the pain mechanisms and is comparable to congenital color blindness and similar conditions.

FROM AUTHORS' SUMMARY.

REGENERATION OF THE MALARIAL SPLEEN IN THE CANARY AFTER INFARCTION
AND AFTER BURNING. W. BLOOM and W. H. TALIAFERRO, J. Infect. Dis.
63:54, 1938.

Infarcts of varying sizes occur sporadically in the spleens of canaries heavily infected with *Plasmodium cathemerium*. They are essentially hemorrhagic and are associated with propagating thrombi in the splenic veins, which generally extend from the capsule toward the hilus. They probably result from the malarial infection, as all attempts to associate them with bacteria, viruses or intravenously injected india ink have failed.

Practically all such infarcted areas become completely regenerated, as ascertained by laparotomy and by microscopic examination of the tissues. (Less than 1 per cent show permanent scars.) The first stage in the repair and regeneration is the appearance of macrophages between the healthy and necrosed tissue. The macrophages arise from the reticular cells, macrophages and lymphocytes of the adjacent healthy tissue and from hematogenous agranulocytes (lymphocytes and monocytes). The inflammatory process continues until a young scar is formed containing many spindle cells (fibroblasts) and macrophages. This scar is then infiltrated with lymphocytes, which migrate from the healthy spleen and blood vessels or arise in situ by transformation of spindle cells (fibroblasts) into large lymphocytes and by proliferation of local medium-sized and large lymphocytes. Nests of proliferating lymphocytes associated with the smaller arteries give rise to new nodules, and the fibroblasts of the scar become the reticular cells of both the red and the white pulp. The venous sinuses of the red pulp arise from capillaries of the scar. The process is the same whether the infarct is small or large but is slower in the completely infarcted spleen, owing probably to the fact that in the latter, as all of the splenic tissue is dead, the necessary cells must be mobilized from the blood.

The same type of complete splenic regeneration follows experimental burning of a tip of the spleen except that the speed of regeneration is slower, owing probably to the absence of the capsule, and there is greater exudation of heterophils during the early stage of the inflammation and of eosinophils during the late stage. that of repair.

FROM AUTHORS' SUMMARY.

EXPERIMENTAL LEAD NEPHRITIS. W. EGER, *Virchows Arch. f. path. Anat.* **299**: 654, 1937.

One series of rats received 1 per cent neutral lead acetate solution subcutaneously. These injections led after a few days to subcutaneous abscesses. The administration of the lead solution was interrupted until the abscesses healed. The longest duration of life was eight months. A second series received subcutaneous injections of a dilute solution of basic lead acetate; the longest duration of life was four and one-half months. In only two of the animals of the series was the kidney macroscopically reduced in size. Tubular nephritis was produced. In this process the formation of intratubular concretions of lead played a part. Glomerular and vascular changes were not observed.

O. T. SCHULTZ.

EFFECTS OF PARENTERAL INTRODUCTION OF PROTEIN AND NONPROTEIN COLLOIDS. H. HEINLEIN, *Virchows Arch. f. path. Anat.* **299**:667, 1937.

The protein substances used in these experiments were caseosan (a product that is essentially a 5 per cent solution of casein), horse serum and killed growths of the dysentery bacillus. The nonprotein materials used were trypan blue, thorium dioxide and colloid preparations of copper, bismuth and silicon. Rabbits were used. The material was injected into the ear vein over a prolonged period at intervals that did not lead to shock. Following the injection of the protein substances, parenchymal damage extending to localized necrosis and final fibrosis was observed in the liver and myocardium. More striking was the inflammatory reaction in the arteries of the lung, heart, kidney and liver. This consisted in subendothelial proliferation of histiocytes, lymphocytic infiltration of the adventitia and fibrinoid swelling of the media. This panarteritis, which may simulate closely the histologic picture of periarteritis nodosa, is similar to the hyperergic inflammatory reaction of specifically allergic animals. It is probably due in each case to the action of protein split products. Following injection of the nonprotein colloids, the inflammatory reaction varied in severity with the toxicity of the substance and the degree of vital storage by the reticuloendothelium. Injury to endothelium caused increased capillary permeability and serous inflammation. Other changes were similar to but not so marked as those elicited by the proteins. Protein split products derived from host tissues by the action of the injected nonprotein material are believed to be a factor in this part of the inflammatory reaction.

O. T. SCHULTZ.

Pathologic Anatomy

VITAL STAINING OF CONNECTIVE TISSUES. L. S. KING, *J. Exper. Med.* **68**:63, 1938.

Trypan blue injected intravenously is bound almost at once by the intercellular connective tissue elements all over the body—by collagen, reticulin and elastic fibers. This union of dye and tissue elements is the factor responsible for the early macroscopic blue color and is antecedent to cellular colloidopexic action. Different specimens of connective tissue differ among themselves in their ability to hold the dye. Diffuse staining of elastic fibers, noted by previous observers, is merely a special example of the general affinity of connective tissue for the dye. The evidence suggests that histiocytes are cells specialized to segregate noxae that become diffusely bound to the intercellular connective tissue matrix.

FROM AUTHOR'S SUMMARY.

EFFECT OF OBLITERATION OF EFFERENT SEMINAL PASSAGES ON THE TESTIS. P. RICHTER, *Virchows Arch. f. path. Anat.* **300**:225, 1937.

The permeability of the efferent seminal passages was investigated in 525 necropsies on adult males in the course of a year. In 5 of the subjects there

was unilateral obliteration of the vas deferens. In 18 there was occlusion of the epididymis, associated three times with obliteration of the vas on the same side. In 5 subjects the epididymis was occluded on both sides, and in a sixth there was occlusion of one epididymis and of the opposite vas. Thirty-five sterile marriages were recorded for the subjects examined; in only 4 of the men whose marriages were sterile was there bilateral occlusion of the efferent seminal passages as the result of previous gonorrhea such that it could cause sterility. This gives an incidence of 10 per cent, as compared with the usually stated 30 per cent, in which bilateral involvement in the male could be blamed for a sterile marriage. In no instance, whether the obliteration was unilateral or bilateral, was any disturbance of spermatogenesis observed unless it was occasioned by the general state or illness of the subject. In unilateral involvement no difference in spermatogenic function was observed in the two testes. In 50 of the 525 subjects spermatozoa were not found in either seminal vesicle, an observation which evidently was the result either of obliteration more distally situated or of chronic inflammation of the vesicles. The presence of spermatozoa in the seminal vesicles of the remaining subjects speaks against the view that the tail of the epididymis is the only place of storage of spermatozoa.

O. T. SCHULTZ.

VENOUS STASIS OF THE MYOCARDIUM. A. THELEN, *Virchows Arch. f. path. Anat.* **300**:243, 1937.

A discussion of the venous circulation of the heart and of the ways in which it may be impeded precedes a report of a study of 110 hearts selected from a total of 1,000. Myocardial venous stasis is evidenced by a red or brownish red color of the muscle, by escape of droplets of blood from the engorged veins of the cut surface and histologically by engorgement of the veins and capillaries. Stasis is brought about by interference with the pulmonary circulation leading to stasis and increased pressure within the right chambers of the heart. With lesser degrees of involvement of the pulmonary circulation, cyanosis of the myocardium may develop acutely from sudden strain of the right side of the heart. Poor circulation through the coronary arteries leads to stasis, and frequently the condition is seen in the myocardium in elderly persons. Hypertrophy of the left side of the heart is not usually associated with passive hyperemia. Passive congestion leads to fatty change and edema of the myocardium.

O. T. SCHULTZ.

FIBRINOID DEGENERATION OF CONNECTIVE TISSUE. E. BAHRMANN, *Virchows Arch. f. path. Anat.* **300**:342, 1937.

In a study of fibrinoid degeneration of inflamed tissues Bahrmann found the silver method of the Oliveira helpful. The process is not the result of swelling and homogenization of the connective tissue, with disappearance of the fibrils, as claimed by Neumann, nor to swelling of the collagenous ground substance, as claimed by Klinge. The fibers become condensed and compressed. This gives them a density on which the optical properties and the staining reactions of both fibrin and the degenerated tissue depend. Other fibers are swollen as the result of imbibition of fluids containing the soluble precursors of fibrin. Both condensation and swelling may be present at the same time, and transition of one process to the other is evident. Separation of the fibrils of the collagenous tissue by the imbibed fluid causes the fibrils to become argentophilic.

O. T. SCHULTZ.

HEPATOSIS AND ICTERUS. LA MANNA, *Virchows Arch. f. path. Anat.* **300**:398, 1937.

La Manna includes under the designation "hepatosis" the various parenchymatous degenerative changes that are not part of the inflammatory reaction

ABSTRACTS FROM CURRENT LITERATURE

The intralobular system of bile canaliculi is considered a differentiated part of the cytoplasm. Nonstainability of the canaliculi is evidence of parenchymatous damage; other evidences are poor staining of the cytoplasm, alterations in the arrangement of the liver cell cords and cytoplasmic inclusions, such as bile or iron pigment granules. Morphologic evidence of parenchymatous damage is not always detectable, even though there are clinical manifestations of dysfunction. Cholangiolosis is a type of hepatosis in which the intralobular system of bile canaliculi is disrupted. Some of the canaliculi may contain bile casts. Cholangiolosis is an important factor in the genesis of icterus. Other factors are dissociation of liver cell columns, dysfunction of the reticuloendothelial system and obstruction.

O. T. SCHULTZ.

MYOEPIThelial PROLIFERATION OF MAMMARY DUCTS. R. GUENTHER, Virchows Arch. f. path. Anat. **300**:448, 1937.

The elongated cells with acidophilic cytoplasm lying between the epithelium and the basement membrane of the small and medium-sized ducts of the mammary gland have been termed by Masson, Krompecher and others myoepithelium. Observation of proliferation of this tissue in a breast led to systematic examination of the breasts of 64 women coming to necropsy at the ages of 12 to 79 years and of tissue from 53 women in whom resection had been done because of cystic disease of the breast (mastopathia cystica chronica). In 12 of the necropsy series and in 13 of the surgical series myoepithelial proliferation was observed. The process bore no relation to cystic disease, although the proliferation was somewhat more marked in cystic disease. It was seen in women at beyond the menopausal age and is held to be one of the several hypertrophic changes that are associated with atrophy of the breast at this period of life.

O. T. SCHULTZ.

ANALYSIS OF SEVENTY CASES OF LYMPHOGRANULOMATOSIS. H. STEPHANI, Virchows Arch. f. path. Anat. **300**:495, 1937.

Stephani presents a statistical analysis of 70 cases of lymphogranulomatosis that were examined post mortem at the Charité, Berlin, Germany, in the years from 1929 to 1936. Of the various lymph node groups, the retroperitoneal was involved in 80 per cent of the cases, as compared with 67 per cent for the cervical group, which is usually held to be the most frequently involved. Of the internal organs, the spleen was most frequently affected in 69 per cent of the cases, the liver next most frequently, in 43 per cent. The lung was involved in 37 per cent—by direct continuity from the mediastinum in 10 and by metastasis in 16 cases. In 3 cases in which there was an association with pulmonary involvement, necrosis had led to cavity formation. The kidney was involved in 17 per cent of the cases—in 8 by metastasis and in 4 by direct continuity. One or both adrenals were affected in 7 cases—only once by metastasis. Gastrointestinal involvement was noted in 23 per cent. Cutaneous lesions were noted in 6 per cent of the cases. In 2 cases the process was held to have been cured by irradiation. The skeleton was affected in 40 per cent of the cases. Bone involvement occurred most often by hematogenous metastasis and infrequently by direct continuity (4 cases). The vertebral column was affected most frequently, in 23 cases, and the femur next most frequently, in 13 cases.

O. T. SCHULTZ.

GENESIS OF CORPORA AMYLACEA OF THE CENTRAL NERVOUS SYSTEM. A. SAXÉN, Virchows Arch. f. path. Anat. **300**:534, 1937.

A controversy exists as to whether the corpora amylacea encountered in the central nervous system are derived from degenerated ganglion cells, axis-cylinders, myelin sheaths or glia. In a previous investigation of the auditory nerve, under-

taken for other purposes, corpora amylacea were frequently seen. In the auditory nerve the bodies are localized to the region of the glial septum between the central and the peripheral portion of the nerve. A systematic study of this portion of the auditory nerve in persons from 50 to 95 years old was undertaken with the aim of determining the origin of the corpora amylacea. In many instances the root of the seventh nerve was also investigated as a control. The bodies appear first as globular swellings of the ends of regenerating axis-cylinders or of split degenerating neurofibrils. The fibrillary glia forms a capsule about each enlargement, which loses its connection with the rest of the axis-cylinder. Proliferated syncytial glia about regenerating axis-cylinders also takes part in the formation of corpora amylacea. Their formation is a phenomenon of senility; local arteriosclerotic changes have an important part in their formation.

O. T. SCHULTZ.

EARLY RENAL CHANGES IN TOXIC NEPHROSIS. C. CORONINI, *Virchows Arch. f. path. Anat.* **300**:594, 1937.

As an early change in toxic nephrosis Coronini describes swelling of the afferent vessel of the glomerulus and of the stalk of the glomerulus. Mesenchymal proliferation was observed in this region. Although the changes described might be considered a part of an inflammatory reaction, he holds them to be noninflammatory—an evidence of mesenchymal injury in toxic tubular nephrosis.

O. T. SCHULTZ.

RELATION OF CHRONIC INTRACRANIAL PRESSURE TO FATTY CHANGE OF THE LIVER. E. J. KRAUS, *Virchows Arch. f. path. Anat.* **300**:617, 1937.

In previous publications Kraus had shown that increased intracranial pressure of sufficiently long duration, due to a wide variety of causes, was associated with hypertrophy of the hypophysis if the integrity of the midbrain had not been destroyed by the pressure. Because of the relation of the hypophysis and midbrain to metabolism he made a systematic study of the liver in 36 cases of chronic intracranial pressure. In 80 per cent of the cases the liver revealed the presence of fatty change. Characteristic of the latter was its central location. The cases studied are divided into nine groups, according to the degree of fatty change and its spread from the central zone to the intermediate and peripheral zones of the lobule. The fat was present in the form of large droplets or in that of very fine droplets; most often large and small droplets were intermingled. In 71 per cent of 33 adults the hypophysis was enlarged. The hypertrophy was not associated with any specific type of cell. The chief cells were most often increased in number, and in males this increase was associated with an increase in the number of basophilic cells. The fatty change of the liver bore no relation to the nutritional state of the patient and was not associated with any of the diseases that usually lead to fatty change. The process is held to be due to the influence of the hypophysis and midbrain on metabolism. In many cases the adrenal cortex was hyperplastic and contained an increased amount of lipoid. This is ascribed to increased formation of corticotropic hormone by the anterior lobe of the hypophysis.

O. T. SCHULTZ.

THE STRUCTURE OF THE BASEMENT MEMBRANE. K. MUTO, *Virchows Arch. f. path. Anat.* **300**:652, 1937.

Muto defines basement membrane as the homogeneous layer which divides connective tissue externally from epithelium and internally from capillaries and muscles. Whether the membrane is fibrillary or homogeneous is controversial. Muto's observations are based on the use of various staining methods, especially the silver impregnation methods. He conceives the basement membrane to be

composed of argentophilic fibrils embedded in a ground substance. The latter may be in the sol state; then it is not visible by any method and may appear as a space in the tissue. Or it may be in the gel state, in which event it is stainable and appears homogeneous or sometimes granular. The size, shape, arrangement and staining properties of the fibrils are not uniform. New formation of the basement membrane was observed in epithelial hyperplasia, both benign and malignant. This may occur by actual new formation of ground substance and fibrils or by rearrangement of the fibrils previously present. Transformation of the membrane may be localized and lacunar. This state may be brought about by liquefaction of the ground substance and rearrangement of the fibrils or by localized disappearance of portions of fibrils and liquefaction of the ground substance. Diffuse transformation of the basement membrane is the result of deposition in the ground substance of amyloid, hyalin or fat.

O. T. SCHULTZ.

Microbiology and Parasitology

FOWL POX VIRUS. G. J. BUDDINGH, J. Exper. Med. 67:921 and 933, 1938.

Intracerebral inoculation of the virus of fowlpox in young chicks produces within from four to five days a disease characterized by drowsiness and somnolence. These symptoms are followed on the sixth and seventh days by spastic paralysis and convulsions. The majority of inoculated chicks die on the seventh or the eighth day. The pathologic lesions are found chiefly in the meninges, perivascular structures, choroid plexuses, paranasal sinuses, mastoid cells, bone marrow of the cranial bones and orbital tissues. No affinity for nerve tissue per se develops. In this environment the virus has high virulence for the epithelium of the choroid plexus and acquires the capacity of infecting cells of mesodermal origin. All infected cells, of whatever origin, undergo a similar structural change: Fowlpox inclusions appear within them, and they become spherical and detached from one another.

The virus has been carried through fourteen successive intracerebral passages. The symptoms and lesions in chicks inoculated with the virus after the fourteenth passage showed no marked difference from those of the chicks inoculated after the first passage. The changes brought about in the virus by the intracerebral environment do not seem to be enhanced by repeated passages.

Intracerebral transfer of this virus in chicks produces marked changes in the behavior of the virus as studied in the chorioallantoic membrane of chick embryos and in the skin of baby chicks. The virus thus propagated shows a great and persistent increase in virulence for epithelial cells, as evidenced by rapid necrosis instead of proliferation and hyperplasia of these cells. It shows also an affinity for cells of mesodermal origin, including endothelial cells of blood vessels, and an increase in affinity for endodermal cells. The intracerebral virus causes a uniform morphologic change in all types of cells in that the infected cells rapidly become spherical, detached and desquamated, this process being followed by necrosis. One intracerebral passage is sufficient to produce this change in the virus.

FROM AUTHOR'S SUMMARY.

EXPERIMENTAL GONOCOCCIC INFECTION IN MICE AND THEIR PROTECTION BY SULFANILAMIDE. A. COHN and L. R. PEIZER, J. Infect. Dis. 63:77, 1938.

The mouse may be used in studying chemotherapeutic effects on the gonococcus. There is only one route so far by which the mouse may be infected, namely, the peritoneal. The important factors in experimental gonococcic infection in mice are the preparation of the mucin emulsion, the addition of the dextrose and the choice of the strain of gonococci. The mice used should weigh about 18 to 20 Gm.

The spread of the infection in the mouse follows multiplication of the organisms in the peritoneum, from which the gonococci invade the blood stream. After removal of the peritoneal focus, the organisms disappear from the blood stream.

In current chemotherapeutic studies on gonococcus-infected mice it was found that feeding of sulfanilamide protects mice only when larger doses of this drug are administered than are necessary for subcutaneous injection. When 25 mg. of sulfanilamide was either fed or injected subcutaneously, an average of 65 per cent of the infected mice were protected.

FROM AUTHORS' SUMMARY.

PULMONARY SCHISTOSOMIASIS. A. F. B. SHAW and A. A. GHAREEB, *J. Path. & Bact.* 46:401, 1938.

In 282 autopsies on Egyptians suffering from schistosomiasis, pulmonary lesions due to ova were found in 33 per cent. The toxic effect of the ovum is shown by necrosis of the tissue in its vicinity. The amount of necrosis varies and possibly depends on the degree of allergy at the time of invasion. The tissues may respond to the presence of ova by reactions other than the formation of tubercles, and it is suggested on the basis of the histologic evidence that the number of ova, reinfection, immunity and allergy may all play a part in determining the type of response. The ova reach the lungs as emboli and become impacted in the arterioles which accompany the respiratory bronchioles, producing a specific actively necrotizing arteriolitis. After necrosis the ovum escapes through the wall of the vessel, and a parenchymatous tubercle forms near the respiratory bronchiole. It is suggested that the ovum secretes an anticoagulant, as it never excites thrombosis.

The number of ova is of primary importance in judging the effect on the pulmonary tissue. In 86 per cent of the cases only a few had entered the lungs, and the only lesions present were parenchymatous tubercles. Embolic ova were rarely seen in these cases. In cases of heavier infection, vascular lesions as well as parenchymatous tubercles were present, and embolic ova were frequent.

Healing of the acute vascular lesions leads to obliterative arteriolitis, often followed by canalization of the occluding tissue. The new-formed capillaries hypertrophy, producing a structure characteristic of pulmonary bilharziasis, to which the authors give the name "angiomatoid." The vascular changes are focal in distribution. They are unassociated with cardiac hypertrophy or signs of congestive heart failure. Massive and repeated infection of the lungs is followed by widespread arterial changes, hypertrophy of the right ventricle and development of the cardiopulmonary features of Ayerza's disease, with death from congestive heart failure. The severity of the disease is largely due to repeated reinfection of healing or healed lesions. The gross appearances of the lungs are specific only when ova of reinfection fail to complete their migration through the thickened arterioles, with development of tubercles in the walls. At a later stage, when the specific lesions heal, only the effects of long-standing arterial obstruction are evident, and to the naked eye the appearances do not differ from those in Ayerza's disease due to other causes. Microscopically, however, the bilharzial origin can be recognized by the characteristic angiomatoid structure, even although all the ova may have disappeared. The lungs are the seat of chronic passive congestion without hemosiderosis. Two and one-tenth per cent of all cases of schistosomiasis and 6.3 per cent of the pulmonary cases are instances of Ayerza's disease of bilharzial origin. Reasons are given for believing that the latter disease is a common complication in Egypt and other countries where bilharziasis is endemic. Ova of *Schistosomum haematobium* (58 per cent) are more common in the lungs than ova of *Schistosomum mansoni* (31 per cent), but *S. mansoni* produces vascular lesions more often (54 per cent) than *S. haematobium* (19 per cent). The reasons for this are considered. Evidence is advanced to show that the passage of the ovum through the wall of the vessel is due to necrosis produced by a toxic action of the embryo, the spine playing little or no

part in the process. It has been shown that the ovum can escape through the wall under conditions in which the size of the vessel and the absence of contractility make it impossible for the spine to exercise the piercing function attributed to it by the mechanical theory. Worms were present in the lungs in 3.6 per cent of the series and in 10.5 per cent of the pulmonary cases. Either *S. haematobium* or *S. mansoni* may occur. The worms reach the lungs by the pulmonary artery and are usually arrested as riding emboli at the bifurcation of a vessel. Although bathed in venous blood, they die rapidly. While alive, they produce no structural changes, but the dead worm is highly toxic, causing necrosis of the artery and acute focal necrotizing pneumonia. Later the pneumonic exudate is absorbed and cicatrized, but the defunct worm becomes calcified and enveloped in scar tissue.

FROM AUTHOR'S SUMMARY.

Immunology

TUBERCULOSIS IN ALLERGIC AND DESENSITIZED GUINEA PIGS. H. S. WILLIS and C. E. WOODRUFF, *Am. J. Path.* **14**:337, 1938.

A study of histologic sections from the lungs of desensitized guinea pigs revealed extensive disease with an overwhelming accumulation of acid-fast bacilli. No definite tubercle formation was seen in sections of the liver and spleen. Lesions occurred in the kidneys and blood vessels of both the desensitized and the control animals. It is suggested that the lack of allergy was responsible for the free and unrestrained growth of tubercle bacilli in the lungs of the desensitized guinea pigs.

FROM AUTHORS' SUMMARY.

ACTIVE IMMUNIZATION OF NURSES AGAINST SCARLET FEVER. E. H. PLACE, *Am. J. Pub. Health* **28**:137, 1938.

Toxin immunization of nurses, when carefully done, largely abolishes evidence of scarlet fever in them during their training in the handling of patients with contagious diseases. There is no evidence that the disease is still occurring in unrecognized form.

FROM AUTHOR'S SUMMARY.

NASALLY INSTILLED POLIOMYELITIS VIRUS. A. B. SABIN and P. K. OLITSKY, *J. Exper. Med.* **68**:39, 1938.

With a method of intranasal instillation of the virus of poliomyelitis that brings about infection of all rhesus monkeys subjected to it, Sabin and Olitsky undertook a study of the fate of nasally instilled virus in normal and in convalescent, immune animals. Control experiments revealed that the nasal mucosa of normal monkeys contained no observable antiviral factors and that when 5 or 10 minimal cerebral infective doses were added to the mucosa, the virus could be detected by the procedure employed. In the olfactory bulbs even a single infective dose could be recovered, since suspensions of both bulbs could be transferred to the brain of a monkey without any loss of material. After nasal instillation in normal monkeys, the virus disappeared quickly (within four hours or less) and could be recovered neither from the excised nasal mucosa nor from the olfactory bulbs during the first forty-eight hours. At seventy-two hours, just before or coincident with the first rise of temperature, it was found in very small amounts in the nasal mucosa and for the first time also in the olfactory bulbs. At ninety-six hours, at least three days before the appearance of nervous signs, while virus continued to be present in considerable amounts in the olfactory bulbs (and presumably elsewhere in the central nervous system), none was detected in the nasal mucosa. In convalescent, immune animals receiving intranasally the same strain of virus which caused the original infection, none could be recovered from the nasal mucosa or central nervous system at four hours, one, two, three, four, five and seven days. The bearing of these observations on the problem of host to host transmission of the virus of poliomyelitis is discussed.

FROM AUTHORS' SUMMARY.

THE IMMUNOLOGIC BEHAVIOR OF SERUM GLOBULIN. J. MARRACK and D. A. DUFF, Brit. J. Exper. Path. **19**:171, 1938.

The behavior of the water-soluble and insoluble fractions of serum globulin and antiserum to whole serum globulin has been investigated quantitatively. The results suggest that these fractions are not present as such in the whole globulin.

FROM AUTHORS' SUMMARY.

COMBINATION OF VACCINIA WITH ANTIVACCINIAL SERUM. M. H. SALAMAN, Brit. J. Exper. Path. **19**:192, 1938.

Suspensions of vaccinal elementary bodies treated with antivaccinal serum suffer a reduction of infective titer. This reduction persists after all free serum has been removed. The reduction of infectivity is not the result of agglutination of the elementary bodies. The results recorded are consistent with the hypothesis which has been advanced for other serum-virus systems, that under constant conditions a given quantity of antiserum inactivates a constant percentage of any dose of virus on which it acts.

FROM AUTHOR'S SUMMARY.

PRODUCTION OF ANTI-N IMMUNE SERUMS IN RATS. S. OLBRICH, Ztschr. f. Immunitätsforsch. u. exper. Therap. **91**:242, 1937.

Rats inoculated with human red cells NO yielded serums agglutinating human red cells in dilutions varying from 1:40 to 1:320. Absorption with red cells MA gave specific and usable anti-N testing fluids. Attempts to produce anti-M serums failed, and, strangely, from serums of rats inoculated with MO red cells, anti-N testing fluids could be prepared by absorption with M red cells. It cannot be decided whether the readiness to produce agglutinins against the property N and the failure to produce them against M are due to the serologic structure of the rat or to the antigenic properties of the human red cell. The rat shows a similar readiness to produce antibodies against the blood group A and not against B.

I. DAVIDSOHN.

DISTRIBUTION OF BLOOD GROUP FACTORS IN THE TISSUES. V. FRIEDENREICH and G. HARTMANN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **92**:141, 1938.

Some persons eliminate their blood group factors in certain secretions (gastric juice, saliva, bile, sperm and urine); others do not. The original explanation of this as due to secretion from the blood is invalidated by the finding in glands and tissues of eliminators of large amounts of blood group substances as compared with traces only or complete absence of such substances in the corresponding tissues of noneliminators. In the former group the largest quantities of the group factors (A, B and O) were found in the stomach, submaxillary glands, pancreas and gallbladder; the smallest amount, in the testicles and in the blood. A calculation showed that the quantity of the group factors in the gastric secretion was five hundred times larger than the quantity available in the blood that reached the stomach during the estimated period. The group antigens are produced in the large glands and enter the secretions.

I. DAVIDSOHN.

SEROLOGIC DIFFERENTIATION OF HOMOZYGOTES AND HETEROZYGOTES IN GROUPS A AND B. P. DAHR, Ztschr. f. Immunitätsforsch. u. exper. Therap. **92**:180, 1938.

Homozygotes of group A₁ have the genotypic formula AA, heterozygotes the formula AO, homozygotes of group B the formula BB and heterozygotes the formula BO. Some normal beef serums have specific anti-O agglutinins; when these serums are absorbed with cells A₁B, the anti-O agglutinins decrease only slightly, while a large majority of red cells of subgroups A₁ and B remove a con-

siderably larger quantity of the anti-O agglutinins, and cells of subgroup A₂ remove still more. The proportion of the A₁ and B red cells which remove a great deal of the anti-O agglutinins to those which remove about the same amount as do the A₁B red cells is similar to the incidence of homozygotes and heterozygotes in blood groups A and B which can be expected according to the formulas of Bernstein. The degree of absorption of the anti-O agglutinins in the beef serum corresponds, according to Dahr, to the quantity of the O factor in the genotypic formula. Dahr advocates further absorption studies with red cells of known genotypic formulas: A₁A₁, A₁A₂, A₁A₃ and BB. I. DAVIDSOHN.

Tumors

EFFECT OF 1,2,5,6-DIBENZANTHRACENE ON THE GROWTH OF BROWN-PEARCE RABBIT CARCINOMA. M. APPEL, A. A. STRAUSS, G. KOLISCHER and H. NECHELES, *Am. J. Cancer* **33**:239, 1938.

In rabbits treated with 1,2,5,6-dibenzanthracene the Brown-Pearce rabbit carcinoma grew more rapidly and metastasized much more extensively than in control animals. The metastases were more numerous, appeared earlier and were found in organs rarely involved in the growth of this tumor, as the spleen, thyroid gland, adrenal glands and myocardium. The proportion of "takes" and the proportion of deaths as a result of carcinomatosis were also increased in the treated animals. The increase in growth of the tumor is attributed to an increase in susceptibility or to a change in predisposition to tumor growth brought about by 1,2,5,6-dibenzanthracene.

FROM AUTHORS' SUMMARY.

CARCINOSARCOMA. O. SAPHIR and A. VASS, *Am. J. Cancer* **33**:331, 1938.

One hundred and fifty-three cases of so-called carcinosarcoma are recorded. A critical review indicates that the carcinosarcomatous nature of these tumors is questionable. Perhaps only 3 or 4 of them may be designated as true carcinosarcoma. A number of observations are given on tumors which were at first thought to be carcinosarcoma but which on more careful examination were interpreted as primary carcinoma. In evaluating the seemingly sarcomatous features of the reported carcinosarcomatous growths, the following four complicating factors which play a role in the alteration of the fundamental histologic appearance of the tumors must be considered: (1) variations of carcinoma cells, some of which assume spindle shapes and may be interpreted as cells of a spindle cell sarcoma, a factor particularly true of "squamous-cell carcinomas with transitional features"; (2) marked anaplasia of carcinoma cells; (3) chronic inflammation which leads to morphologic changes of tumor cells or produces much connective tissue, which may be regarded as part of a malignant connective tissue tumor, or provokes a lymphocytic reaction, sometimes taken as the lymphosarcoma component of the tumor, and (4) invasion of a benign connective tissue tumor by carcinoma. Other instances of so-called carcinosarcoma are believed to be cases of sarcoma invading normal or metaplastic epithelial structures, the latter being interpreted as the "carcinomatous" elements.

FROM AUTHORS' SUMMARY.

HIGH INCIDENCE OF SPONTANEOUS MAMMARY TUMORS IN ALBANY STRAIN OF RATS. W. R. BRYAN, G. H. KLINCK JR. and J. M. WOLFE, *Am. J. Cancer* **33**:370, 1938.

A high incidence of tumors and low fertility have been noted simultaneously in females of the Albany strain of rats during the past eighteen months. The tumors have for the most part been of the benign fibroepithelial type. In addi-

tion, there have been observed: adenocarcinoma, adenoma and fibroma. Three of the fibroepithelial tumors displayed the characteristic picture of intracanalicular adenofibroma.

FROM AUTHORS' SUMMARY.

USEFUL METHODS FOR ROUTINE EXAMINATION OF BRAIN TUMORS. N. C. FOOT, *Am. J. Path.* **14**:245, 1938.

Foot states that thus far diagnoses have been made of astrocytoma, polar spongioblastoma, glioblastoma of the multiform type, oligodendroglioma, and glioblastoma arising from the adrenal region, with origin in the sympathetic nerves. The last-named tumor would have passed as an unusual form of carcinoma; in fact, it did until silver impregnations demonstrated the structure of the multipolar astrocytes contained in it. Their processes were practically unnoticeable in the sections prepared with the hematoxylin-eosin stain but were better shown in the trichrome sections, which led Foot to try silver impregnation. With this they came out excellently. There is no reason, he says, why the combination of the methods set forth in this paper should not be equally good for ependymoma and those tumors having cells of a more immature type—so-called medulloblastoma and neuroepithelioma. He has found them excellent in the case of meningioma. Naturally, they are also applicable to tumors of peripheral nerves, with which they give splendid results, affording a means of accurate diagnosis.

FROM AUTHOR'S SUMMARY.

COMEDO CARCINOMA OF THE BREAST. D. LEWIS and C. F. GESCHICKTER, *Arch. Surg.* **36**:225, 1938.

Comedo carcinoma occurs in two rather characteristic forms: diffuse and localized. The diffuse form presents some of the clinical features peculiar to intracanalicular myxoma. It grows slowly and involves the greater part of the breast; no isolated tumor can be palpated in the enlargement. Despite the size of the growth, there are frequently no palpable lymph nodes. Small elevations in the skin may be found, which are caused by the protrusion of the epithelial plugs within the ducts. Not infrequently a discharge from the nipple is noted.

The localized form of the tumor is small, from 1 to 3 cm. in diameter. It is usually situated at the margin of the areola just beneath the skin and is freely movable. The axillary nodes as a rule are not involved. Not infrequently there is a yellowish or watery discharge from the nipple. The affected breast is slightly larger than the uninvolved breast. The tumor differs from intracystic papilloma and blue dome cyst in that it is relatively harder and more irregular.

The age incidence of comedo carcinoma corresponds to the age incidence of other forms of carcinoma of the breast. The location of the comedo carcinoma suggests origin in the larger ducts. Retraction or fixation of the nipple occurs often, and occasionally the patient complains of burning and itching of the nipple, a symptom more common in Paget's disease. The tumor is usually located near the skin, and atrophy of the overlying fat and dimpling of the skin occur. The tumor remains movable. Even when the growth is larger than a large grapefruit and involves almost the entire breast, there will be no fixation to the wall of the chest. Several tumors may be found in the same breast. Of all the forms of carcinoma of the breast, comedo carcinoma offers the most favorable prognosis. Five year cures were obtained in 85 per cent of the patients. The majority of the patients living more than five years after complete operation have remained well for ten years or more.

TRANSMISSION OF THE ROUS FILTERABLE AGENT TO CHEMICALLY INDUCED TUMORS. E. MELLANBY, *J. Path. & Bact.* **46**:447, 1938.

When a fowl carries a tumor of the type induced by chemical agents, such as dibenzanthracene or tar, and at the same time another tumor of the Rous

type, i. e., of the type produced by a filtrable agent, the Rous factor passes into the chemically induced tumor but leaves it apparently unaffected. If the cells of such a chemically induced tumor are injected into a fowl, they produce a tumor of a structural type which can be propagated further only by inoculation of cells. If a cell-free filtrate of such a chemically induced tumor in a fowl bearing also a Rous sarcoma is injected into other fowls, it produces, if it produces a tumor at all, a tumor of the Rous type. In two of many experiments cell-free filtrates made from the second generation of chemically induced tumors—i. e., tumors which themselves had no association with a Rous sarcoma in the same fowl—produced Rous sarcoma (third generation) which could be propagated further by a cell-free filtrate. Cells of these chemically induced tumors, however, produced other (third generation) tumors of the same type as those originally chemically induced and these gave no evidence of containing the Rous agent. In two experiments second generation dibenzanthracene tumors of fowls regressed and new tumors grew in their place. These new tumors had the character of the Rous sarcoma and could be readily propagated by cell-free filtrates. There is no good reason to believe that the presence of the Rous agent actually made the dibenzanthracene tumors regress, as such regression may occur without the presence of Rous sarcoma in the same bird. Injecting the Rous filtrable agent into a dibenzanthracene tumor will not make it regress but may produce Rous sarcomatous tissue in it. It appears, however, that when regression takes place, the Rous agent exerts its effect and produces a second Rous tumor replacing the dibenzanthracene tumor.

FROM AUTHOR'S SUMMARY.

PREVENTION OF MAMMARY CANCER IN MICE BY THYROTROPIC PITUITARY HORMONE. W. CRAMER and E. L. HORNING, *Lancet* 1:72, 1938.

The thyrotropic principle of the pituitary has been used to prevent the development of mammary cancer in cancer-susceptible mice. The thyrotropic principle has also been used to prevent the changes in the pituitary and mammary glands of male mice caused by estrogen. These observations may prove of value in the treatment of cystic disease of the breast supposedly caused by estrogen.

CANCER IN MADAGASCAR. G. MOUSTARDIER, *Bull. Assoc. franç. p. l'étude du cancer* 27:24, 1938.

Cancer is not as infrequent among the natives of Madagascar as was claimed by some previous writers. The author studied 87 tumors in eighteen months, all of them among the natives. Twenty-five were benign; 14 of these were epithelial, 9 of connective tissue origin and 2 mixed. Sixty-one were malignant: 45 of these were carcinoma, 11 sarcoma, 2 melanoma, 1 glioma, and 2 were embryonal tumors. The absence of tumors of the lips, tongue, mouth and pharynx was the more striking in view of the fact that such tumors are frequent in Europeans and are easily detected. Syphilis and smoking and chewing, which are commonly held responsible for some of these growths, are very prevalent on the island. The proportion of cases of sarcoma to cases of carcinoma (1:4) was higher than among the white races. The natives rarely reach the advanced age in which cancer is prevalent. Women were afflicted more often (37) than men (24); this is due to the great frequency of cancer of the breast and of cancer of the uterus.

I. DAVIDSOHN.

A MELANOTIC TUMOR IN A FISH. R. P. DOLLFUS, J. TIMON-DAVID and M. MOSINGER, *Bull. Assoc. franç. p. l'étude du cancer* 27:37, 1938.

Melanotic tumors occur quite frequently in fish; in a series of L. Thomas, they were present in 15 (57 per cent) of 270 tumors. The one reported now differed in certain essentials from all previously reported examples of melanoma. In a fish belonging to the species *Epinephelus gigas* many tumor nodes were seen, the

largest the size of a walnut. Most of them were on the gills, but no part of the body was free from them, including the liver, spleen and peritoneum. Histologic preparations showed epithelial masses, mainly in the form of distinct round nodules, some with a peripheral zone of compressed cells. Degenerative changes were abundant and varied. Massive accumulations of a brown granular pigment were seen. They seemed to be of a degenerative nature, but in the hepatic and splenic metastases the melanotic deposits were similar to those seen in true melanotic tumors. The authors believe that this is essentially an epithelial tumor the cells of which have acquired melanogenic properties secondarily.

I. DAVIDSOHN.

TISSUE CULTURE OF LYMPHOGRANULOMA (HODGKIN'S DISEASE). R. MEIER, E. POSERN and G. WEITZMANN, *Virchows Arch. f. path. Anat.* **299**:329, 1937.

In cultures lymphogranulomatous tissue grows more rapidly and more actively than normal lymphoid tissue in vitro. Fibrocytes grow out from the explant and produce a fibrillated zone similar to that of normal lymphoid tissue. In this zone are small lymphocytes and larger round cells with granular cytoplasm. The latter are held to be pathologic large lymphocytes. There also appear in the culture many large giant cells, which are often multinucleated. They are at first homogeneous and optically indistinct but undergo a sudden transformation which makes them less homogeneous and more readily visible. These cells are like the Sternberg-Reed giant cells of lymphogranulomatous tissue, but the authors do not definitely establish the observation that the tissue culture giant cells and Sternberg-Reed cells are identical. When normal lymphoid tissue and lymphogranulomatous tissue were grown side by side in the same culture flask no formation of giant cells by or from the normal tissue was observed. The formation of the giant cells in tissue culture is a fixed and irreversible property of the cells and is characteristic of lymphogranulomatous tissue. It is a property similar to or identical with that of tumor cells. The authors think that the giant cells of the tissue culture are derived from endothelial cells.

O. T. SCHULTZ.

BENIGN BRONCHIAL TUMORS. H. HAMPERL, *Virchows Arch. f. path. Anat.* **300**: 46, 1937.

In 1931 Geipel described as benign basal cell epithelioma 2 bronchial tumors and collected reports of 6 similar benign tumors from the literature. Hamperl describes minutely the gross and microscopic appearances and the clinical manifestations of 9 benign bronchial tumors. These with Geipel's 8 and others described in the literature bring the total to 32. Most of them occurred in persons under 50 years of age. There has usually been a long history of respiratory difficulty, indicating that the tumors usually arise at an early age. The usual clinical findings are those of localized bronchiectasis. The tumor may grow into the lumen as a polypoid growth or may grow within the wall of the bronchus, bulging either outward or inward. In 2 of Hamperl's tumors the histologic picture was identical with that of mucus-secreting cylindroma, such as occurs especially in the salivary glands. The author derives these tumors from mucus-secreting cells of the bronchial mucosa. Each of the other neoplasms had an invasive type of growth within the bronchial wall. They were, however, only locally invasive, did not invade surrounding tissues and did not metastasize. They were composed of solid cords and masses of cells of epithelial type, some tall, of the palisade type, others polyhedral. They also contained cells of the oncocyte type, which Hamperl has previously described in a variety of aging parenchymatous cells. Some of the cells secrete mucus, and some are arranged about narrow glandular lumens. Hamperl considers the structure and morphologic features of these tumors identical with those of the gastrointestinal carcinoid as described by Masson. He therefore terms them bronchial carcinoids. Hamperl admits the theoretic possibility of the develop-

ment of malignancy in the two types of bronchial tumor described, but thus far satisfactory proof of their malignancy has not been adduced. The importance of clinical diagnosis, by roentgenographic, bronchoscopic and histologic examinations, is stressed.

O. T. SCHULTZ.

FORMATION OF RUSSELL BODIES IN PLASMACYTIC MULTIPLE MYELOMA. K. APITZ, *Virchows Arch. f. path. Anat.* **300**:113, 1937.

Different kinds of cellular degeneration products occurring in a variety of cells have been called Russell's bodies. Apitz insists that the typical fuchsinophile body is formed only in the plasma cell. The latter must have the morphologic appearance described by Unna and must give the characteristic pyronine-methyl green staining reaction. In a cytologic study of material in 14 cases of multiple myeloma, the formation of Russell's bodies was observed in 4. In 2 instances the cells were typical plasma cells, whereas in 2 the cells were immature. The presence of fuchsinophile bodies in the latter establishes the plasmacytic character of these tumors and is considered proof that plasma cells may differentiate from myeloblasts. The formation of Russell's bodies within the nucleus is described by Apitz. Neither chromatin nor nucleolar substance takes part in their formation. Russell's bodies are held to be the result of abnormal intracellular metabolism of protein.

O. T. SCHULTZ.

SIMILARITY OF CORTICAL GLIOMA AND PIAL MENINGIOMA. H.-H. MEYER, *Virchows Arch. f. path. Anat.* **300**:296, 1937.

Four cases are described in which it was impossible to determine from the gross characteristics whether a tumor was a glioma that originated in the cortex of the brain and invaded the meninges or a meningioma that originated in the pia-arachnoid and invaded the brain. For meningioma the author prefers the term "arachnothelioma." Microscopic examination after the use of special staining methods established that the tumors described were gliomas. One was astrocytoma and one astroblastoma. Two were glioma multiforme. The gross similarities and microscopic differences of cortical glioma, meningioma and meningeal glioma are discussed.

O. T. SCHULTZ.

Medicolegal Pathology

THE MEDICOLEGAL EXAMINATION OF HAIRS. B. M. VANCE, *New England J. Med.* **218**:914, 1938.

In general, the evidence which results from examinations of hair must be regarded as confirmatory. It should never be used unsupported by other proof. The circumstances in each case must determine how valid will be the facts elicited by examination of the hair. The most fitting person to perform examinations of hair is a graduate of a medical school or a biologist of wide experience. The study of hair is a branch of histology of such complexity that long and painstaking application is required to master it. It is important that the scientist engaged in this pursuit should prepare numerous specimens of human and animal hairs, both in whole mount and in cross section and that he should make photomicrographs of the most typical specimens for the purpose of ready comparison.

FROM AUTHOR'S SUMMARY.

PATHOLOGIC-ANATOMIC STUDIES OF SO-CALLED DURET-BERNER HEMORRHAGES. B. DAHL, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **29**:366, 1938.

The investigations reported indicate that a deadly blow on the head results in immediate general distention of the veins and venules, the circulatory disturbances leading to edema in the inner organs. In the lungs and mesentery hemorrhages

occur. Simultaneously with the circulatory disturbances or even shortly before their onset, respiration ceases and cardiac function becomes superficial, rapid and irregular, and eventually stops. Such alterations occurring in death agony from other causes set in slowly, but in severe injuries of the head they occur almost instantaneously. The tonus of the blood vessels, especially that of the abdominal veins, disappears rapidly, and blood accumulates in these veins as the arteries and capillaries contract and empty themselves. Similarly, blood collects in veins of the brain, subjecting them to the possibility of rupture, especially on the cerebral surface and in the spongy subependymal tissue. These hemorrhages, therefore, are not the cause but the result of death.

There is no destruction of ganglion cells following the rupture of agonally distended veins. Agonal hemorrhages are found regularly in the brain, in the pia and under the ependyma of the ventricles, also in the fourth ventricle. In hemorrhages of the fourth ventricle (Berner hemorrhages) it is impossible to say whether death resulted from trauma of the head or not.

GEORGE J. RUKSTINAT.

CUTANEOUS MARKINGS FROM PLUNGES INTO WATER. A. PONSOLD, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **29**:408, 1938.

Definitely outlined longitudinal light streaks, bordered by raised red ridges, have been noted on the legs of persons striking water after jumping from high bridges. Sometimes the ridges are studded with hemorrhages. Similar but round spots may be found on the buttocks. In persons who survive such accidents, the condition increases in intensity for twenty-four hours, then gradually subsides. In about four days the skin changes vanish. Balázs expressed the belief that the alterations in the skin result from pressure by articles of clothing. Other observers were convinced that the longitudinal stripes are a projection of the long bones on the surface of the skin. There is anemia at the site of impact because the bones exert a pressure against the skin toward the outside. Because of local anemia in the zone of compression, the adjacent vessels are overdistended and produce the red ridges. The red spots on the buttocks are projections of the foramina obturata. The shift of blood is a vital or agonal reaction and remains when death occurs quickly. The skin changes offer a method of determining the site of impact. Ponsold found the same dermal patterns on the body of a woman who fell from a third floor window to a brick sidewalk.

GEORGE J. RUKSTINAT.

THE IDENTIFICATION OF PERSONS BY THEIR BITES. BUHTZ and EHRHARDT, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **29**:453, 1938.

Teeth, because of their hardness, usually are well preserved. Their characteristic arrangement, therefore, may provide identifying marks. The physiologic evolution of teeth furnishes the best gage of age up to 16 years. Later in life, the wear of teeth surfaces, the increased calcification of the root canal and the recession of the alveolar processes are roughly indicative of age. The smaller size of women's teeth furnishes a suggestive but not infallible guide to age. Caries and discolorations of a characteristic type appear in candy makers, chemical workers and metal workers.

The identification of a person from his bite is facilitated when gross anomalies are present in the position, form or number of his teeth. The records of dentists frequently are helpful, since they definitely localize fillings, crowns, bridges or extractions. Burglars have been identified by the impressions their dentures left on food in the place they burglarized.

The methods employed to identify a person who has bitten another have heretofore not proved dependable. The authors believe they have a method which overcomes many of the difficulties. First, they take photographs of the bites in the victim's skin by side illumination and accurately measure distances from the lens,

etc. Second, they make use of a phantom arm made of wood and covered with rubber. This is coated with an egg and flour batter, and the bites of suspected dentures on this arm are compared with the photograph of the wound of the victim. Third, diapositive films are made of the suspected teeth. The films may be superimposed on the original picture or laid on white paper, for comparison.

GEORGE J. RUKSTINAT.

THE MEDICOLEGAL SIGNIFICANCE OF THE CUTANEOUS ELASTIC FIBER SYSTEM.

S. ÖKRÖS, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **29**:485, 1938.

Ökrös fixes skin in a stretched state so that the elastic fibers will be straightened and occur at regular intervals. The elastic fiber system of the skin has a static portion connected with the collagenic fibers and a parenchymatous part enmeshing the sweat glands, nerves and blood vessels. Because of the stocking-like investment of the papillae of the skin the subpapillary elastic fibers do not straighten and therefore do not lend themselves to study.

In wounds produced during life, shortly after death and at periods up to ten days after death the changes that occur in the deeper layers of the cutis are constant. In stab wounds produced during life the meshes of the elastic net are torn, especially in the corners of the wound. The individual fibers are split, wound spirally and in places tied in knots. In the outer part of the wound oral swellings occur on the meshwork. These changes are proof that elastic fibers torn or cut during life retract, roll together and twist. Experimental wounds produced on the same body ten hours post mortem showed the elastic fibers retracted from their wound margins, but swelling, spiraling and twisting were absent. A wound made ten days after death showed only a little retraction of the fibers.

In bullet wounds there are discontinuity of the fibers and shattering of tissue, due to explosive action. At the moment of entrance into the skin a bullet transmits its enormous kinetic energy through the tissues so that there is a rupturing of the meshes not only in the wound but at a considerable distance from it.

GEORGE J. RUKSTINAT.

TRAUMATIC PNEUMOPERICARDIUM. O. BERNER, *Virchows Arch. f. path. Anat.* **299**:751, 1937.

A 4 year old lad who had been struck by an automobile died within ten minutes after reaching a hospital. A moderate degree of subcutaneous emphysema was noted, but this had disappeared at the time of necropsy by the medical examiner, twenty hours after death. There was no emphysema of the mediastinum. The right pleural cavity contained blood and air, and the lung was ruptured posteriorly. The pericardial cavity contained air. There were no adhesions between the pleura and the lung at the site of rupture of the lung. The explanation offered is that the injury caused pneumothorax and compression of the chest, which forced air through the parietal pleura, leading to subcutaneous emphysema, and through the posterior part of the pericardium, where a rent 2 cm. long was found. There were no rib fractures that might have caused emphysema.

O. T. SCHULTZ.

Society Transactions

NEW YORK PATHOLOGICAL SOCIETY

ALFRED PLAUT, *President*

Regular Monthly Meeting, Dec. 22, 1938

ROBERT A. MOORE, *Secretary*

CAVERNOUS TRANSFORMATION OF THE PORTAL AND SPLENIC VEINS. AMOUR F. LIBER and CHESTER R. BROWN.

An apparently healthy white man, 20 years old, suddenly began to have hematemesis, which repeatedly seemed to threaten his life. One year before death he had pneumococcic lobar pneumonia. The spleen became palpable. Anemia and thrombopenia were reported. Osteomyelitis of the tibia appeared. No other focus of infection could be discovered. Following splenectomy there was no hematemesis for five months, but ascites developed (3,500 cc. of abdominal fluid). Omentopexy was performed. During this operation there was a "matted feeling" about the portal vein. The ascites disappeared promptly, but the patient succumbed to renewed hematemesis fourteen months after the onset of the symptoms.

The spleen weighed 530 Gm. Sections near the hilus showed localized thickenings of the intima of the large veins. Liver tissue removed at the first operation was normal. A specimen of liver taken for biopsy at the second operation revealed lymphocytic infiltration and mild patchy fibrosis of the portal spaces.

Necropsy revealed large varices in the diaphragmatic portion of the esophagus. About the entire stump of the splenic vein and the initial 2 cm. of the portal vein was a large, fairly firm, very elastic mass of brownish pink tissue containing innumerable vascular spaces of all sizes, from a diameter of 5 mm. down to the limits of gross visibility. From several of these cavities small phleboliths could be shelled out. This spongy mass measured about 5 cm. vertically and 15 cm. transversely. It extended upward beneath the parietal peritoneum of the lesser sac to the level of the terminal portion of the esophagus, which it partially surrounded. The interior of the portal vein presented several small folds in the intima, which formed a loose network in the proximal centimeter of the vein, where the lumen was moderately narrowed. The splenic vein showed the same type of trabeculation. The intimal folds became more and more numerous and extensive toward the proximal end of the splenic vein. In many places the lumen was subdivided into four or five channels. Microscopically the spongy perivenous tissue revealed innumerable veins of varied sizes and irregular outline. Many contained thrombi in all stages of organization. Between the channels were thick septums of connective tissue, very rich in elastic fibers, fat, nerves and small blood vessels of normal appearance. The intravenous trabeculae were made up of long bands of collagen and fibroblasts, between which were fine elastic fibers and numerous foamy lipophages containing isotropic material staining with scarlet red. At the point of implantation of a trabecula the internal elastica of the vein bulged out but was not interrupted. The underlying muscle layers were dissected by fibrosis. The liver showed very slight, patchy lymphocytic infiltration and fibrosis of the portal spaces. Near the base of the posterior aspect of the left lung was a subpleural mass measuring 3 cm. in diameter and having exactly the gross and microscopic appearance of the periportal tissue. This case poses once more the much discussed question whether cavernous transformation of the portal vein is a congenital anomaly or a canalized thrombus and collateral circulation. The presence of cavernous tissue in the lung suggests multiple vascular malformation. The lack of a favorable effect of splenectomy is of interest, since thrombocytopenia, which Klemperer (ARCH. PATH. 6:353, 1928) stated is a contraindication to splenectomy, was not present in this case.

A second case was one of portal thrombophlebitis with multiple abscesses of the liver and portal cirrhosis. There was chronic pulmonary tuberculosis. The portal vein was divided into two unequal channels by a longitudinal septum. To the distal margin of the septum was attached a thrombus, well organized at its base but fresh and suppurating distally. The microscopic structure of the septum was similar to that of the trabeculae in the first case. An additional feature was patchy calcification. Here the relations suggest strongly that the single septum was a preexisting anomaly on which the thrombus was grafted. A similar septum without any thrombus was observed in the femoral vein by Gibson and Franklin (*J. Anat.* 72: 128, 1937). Klemperer reported 23 cases of portal cavernoma, including a case of his own. The first case in his series was that of Köbrich. An older case has been found, reported by Verneuil (*Bull. Soc. anat. de Paris* 28:246, 1853). Since Klemperer's report, 8 cases have been published in addition to those reported here.

Dr. Maurice Richter supplied some of the material for the first case report.

BRONCHIOLAR NECROSIS IN THE NEWBORN. SHELDON A. JACOBSON.

A full term boy was born spontaneously at 2:40 a. m. He was given routine care and cried lustily. At 6 a. m. his general condition was good. Later in the morning, however, cyanosis supervened, with labored respiration and retraction of the episternal notch. The diagnosis of atelectasis of the left side was made.

Despite the use of an oxygen tent and injections of alpha lobeline and epinephrine hydrochloride, the infant became progressively feebler, and he died twelve hours after birth.

Autopsy disclosed atelectasis of both lungs. Pieces cut from different parts of the lungs sank in water. Microscopic section showed most of the pulmonary tissue collapsed. In the alveoli were many squamous epithelial cells and other debris. The respiratory and terminal bronchioles showed widespread necrosis of their walls, but there was no necrosis of the adjacent areas of the lungs. There was no indication of an exudative or other reaction.

The localization of the lesions and the absence of cellular infiltration speak against vascular occlusion or infection as etiologic factors. It is postulated that in the course of prenatal respiration (the occurrence of which is established by the intra-alveolar debris) the infant aspirated some of its own activated gastric or intestinal juice, which led to lysis.

DISCUSSION

PAUL KLEMPERER: Was there fat in these bands? Did you stain for fat?

SHELDON A. JACOBSON: Fat staining was not done. There were no clear spaces.

PAUL KLEMPERER: I ask because I am not quite clear as to whether the changes observed by Dr. Jacobson are identical with the hyaline bands which were described in the lungs of the newborn in a recent article. The author described hyaline bands in the newborn in 4 cases, and the bands in the illustration not only resemble very much the hyaline bands which are found in cases of rheumatic fever but also in cases of chronic passive congestion of severe degree. His explanation was that aspiration of amniotic fluid is responsible. However, what Dr. Jacobson assumes is, I think, possible. I remember an infant who died a few weeks after birth, following severe hematemesis, and there were similar lesions, but these were actually necrotic lesions and one could see aspirated gastric contents in this case.

IRVING GRAEF: I should like to confirm what Dr. Klemperer has just said and, in addition, mention the excellent work of Farber on hyaline membranes in the lungs of newborn infants. Before Farber, Johnson and Meyer described these findings in the lungs, together with typical epidermal cells, vernix caseosa and meconium, as commonplace in newborn and stillborn infants. The membrane

shown here resembles the hyaline membrane mentioned. I am not certain whether or not necrosis takes place beneath these membranes, but I have seen it quite frequently without any inflammatory response. It is possible that, since the vernix is lipid, some lipolysis may take place and lead to chemical inflammation. Since the newborn infant's gastric juice is virtually free from hydrochloric acid or lipase, it is unlikely that gastric contents play much of a role in pulmonary inflammation, when it is present. It seems to me that in cases of congenital pneumonitis more attention should be paid to the condition of the amniotic fluid and membranes. Often the physician studies the infant carefully and fails to go back to the study of the amniotic membranes as the possible source of infection or of leukocytes.

ALFRED PLAUT: I agree with Dr. Klemperer and Dr. Graef that in all probability what Dr. Jacobson has shown is the same thing as the so-called hyaline layers. My colleagues and I see them quite frequently in our material from newborn infants, and, if I remember correctly, there is no direct connection with any pathologic condition in the mother. We also have seen them in the lungs of several adults, but never in the lungs of patients with rheumatic fever. It probably is just a coincidence that the membranes in the lungs of persons dying of hydrochloric acid poisoning are so very similar to these. Probably the two lesions are morphologically identical but of different genesis.

PAUL KLEMPERER: I am very glad that Dr. Plaut says he did not find these hyaline layers in the lungs in rheumatic fever. I have been trying to find them, and it may be recalled that a year ago there appeared an article by Mason in which he claimed these hyaline membranes were characteristic of rheumatic fever. I never believed it, but I must confess I have seen them in the lungs in rheumatic fever. However, I do not think they are characteristic of rheumatic fever but are characteristic of a peculiar stage of chronic edema and inflammation in the lung in rheumatic fever. It interests me that Dr. Plaut has not seen these hyaline membranes in the lungs in rheumatic fever.

OSSEOUS FINDINGS IN CHRONIC RENAL INSUFFICIENCY IN ADULTS. ARTHUR M. GINZLER (by invitation) and HENRY L. JAFFE.

It is known that the parathyroid glands become enlarged secondarily in so-called renal rickets of children and in chronic renal insufficiency of adults. Our post-mortem observations confirm this knowledge. Furthermore, there is experimental evidence to support the hypothesis that the parathyroid hyperplasia is caused by the hyperphosphatemia that is a feature of these conditions.

The pronounced osseous changes occurring in cases of renal rickets are well known and have been adequately described. Extensive osseous changes, have also been observed in a few cases of chronic renal insufficiency, in which they have usually been denoted as "generalized osteitis fibrosa." In these cases, there was pronounced enlargement of the parathyroids, such as one sees also in cases of renal rickets. Altogether, the osseous findings in these cases in adults may be regarded as the adult equivalent of those noted in cases of renal rickets of childhood. In the presence of such pronounced osseous changes, secondary hyperparathyroidism can safely be inculcated as a contributory causal factor.

The cases with which we are concerned here, however, are those of chronic renal insufficiency in which there are bone changes not clearly ascribable to parathyroid hyperfunctioning, even though associated with some degree of parathyroid enlargement or at least microscopic hyperplasia. Our observations in regard to these cases may be summarized as follows: The bones, though usually not altered grossly, often reveal on microscopic examination mild but clearcut fibroporotic changes in the spongiosa. In these cases, the spongy trabeculae show scattered resorption lacunae containing osteoclasts and connective tissue, and some of them may also present, here and there, deposits of new bone. Occasionally—and specifically when the renal insufficiency has been very protracted—the bones will be found even grossly altered. In these cases, the spongiosa is close meshed, and the trabeculae are thickened and distorted, so that altogether the condition

amounts to osteosclerosis. The microscopic observations indicate that the osteosclerosis has developed through gradual accretion of new bone, despite the alternation of reparative with resorptive processes that must have been going on for a long time.

Only Rutishauser and his associates have described osseous changes sometimes rising to the level of osteosclerosis in patients in whom secondary hyperparathyroidism was probably not an important factor in the development of the bone lesions. There is no experimental proof that the parathyroid hyperplasia in these cases is accompanied by hyperfunction. This fact, together with the mildness of the hyperplasia in our patients, convinces us that the osseous changes described are probably not caused by the action of increased circulating parathyroid hormone on the bones. This conviction is strengthened by a comparison of these osseous changes with those in Recklinghausen's disease. As noted, an increase in circulating parathyroid hormone does seem to be a complicating factor in patients with pronounced enlargement of the parathyroids. In regard to the cases under discussion here, however, we feel that the osseous changes resulted from the chronic acidosis due to the renal damage, and specifically that demineralization of the skeleton followed the forced excretion of fixed base which is necessary to eliminate the acid end products of metabolism.

DISCUSSION

PAUL KLEMPERER: These bone changes in chronic renal insufficiency have been noted by me for years. My associates and I have found the condition most striking in patients with malignant nephrosclerosis. I do not remember whether these patients had a longer period of renal insufficiency than usual. It is interesting that malignant nephrosclerosis, which is not an instance of long-standing renal insufficiency, should give rise to the same changes as illustrated here in chronic renal insufficiency.

H. L. JAFFE: I think it is worth emphasizing that the osseous changes described (including the osteosclerosis) are not detectable roentgenologically in the living subject. Even when autopsy showed advanced osteosclerosis, the sagittally sectioned vertebral column when roentgenographed did not show any clearcut modification of the normal roentgen picture.

ARTHUR M. GINZLER: We saw these changes in 1 of 3 cases of malignant nephrosclerosis examined, but it was our impression that the changes were most commonly observed in cases of chronic pyelonephritis secondary to prostatic obstruction, in which, perhaps the conditions for the development of long-standing renal insufficiency were most favorable.

HISTOGENESIS OF MAMMARY CARCINOMA: A STUDY BASED ON KEY BLOCK SECTIONS OF THE WHOLE GLAND. HAROLD KOPPELMAN (by invitation).

An adaptation of Fraser's key block method (*Surg., Gynec. & Obst.* 45:266, 1927) was used in a study of 8 cases of cancer of the breast, under the direction of Dr. Irving Graef. Each complete breast dissected was cut into serial sections 0.5 to 1 cm. in thickness, and after fixation an average of 34 key blocks were cut for microscopic study, their locations being marked on a drawing or photograph of the gross specimen for orientation of the features observed. For each specimen a schematic serial reconstruction was drawn, illustrating the gross anatomy of the tumor as checked by microscopic observation. This method provided a broad yet quite detailed view of each gland and yielded a series of observations which usually escape notice in the ordinary random review of breast material.

The origin was in each case traceable to duct epithelium at some point confined within normal boundaries, usually an expanded cystic papillary lesion. Secondary spread occurred both within the ducts and through their walls. Multiple foci of origin were observed in 1 case and suggested in 3 others. Intraductal spread extended in 1 case for a distance of 5 cm. and was observed in 3 others at some distance from the primary growth. This mode of spread also accounted for the involvement of lobular (acinar) epithelium which occurred in 6 cases.

Invasive spread from the ducts outward was easily demonstrable, but invasion from cancerous acini was doubtful. The morphologic aspects of the invasive cells

gave no clue as to their origin from ducts or acini. Local invasion was often seen to be propagated by permeation through lymphatics, often periductal and perilobular in location, accounting for the common finding in random sections of nodules of invasive growth adjacent to normal ducts and for the equally common occurrence of invasion of preexisting lobules sparing the epithelium of terminal ducts and acini. So-called histologic types of breast cancer, such as medullary, scirrhous and adenoid, appeared to be merely different types of invasive spread, coexistent in most of the cases, and probably dependent for their appearance on factors of pressure from the soil the cells invade. These histologic features, as well as the factor of anaplasia, which was seen to vary considerably in different areas, appeared to have no consistent correlation with prognosis.

Clinically unsuspected multiple benign neoplasia was observed in 7 of the 8 cases, including papillary growths, cyst formations, adenoma and fibroadenoma, but only the papillary growths (excluding papillary cysts of the so-called sweat gland type) seemed to be related to the origin of carcinoma. While the lesions of chronic cystic hyperplasia were present in 6 cases, in only 1 was malignant extension traceable to the papillary neoplasia, and in that case multiple foci seemed probable.

The observations in this small series are consistent with the concept of the histogenesis of mammary carcinoma evolved from the similar but much more extensive researches of Cheatele (Cheatele, G. L., and Cutler, M: *Tumors of the Breast: Their Pathology, Symptoms, Diagnosis and Treatment*, London, J. B. Lippincott Company 1931), of Dawson (*Edinburgh M.J.* **40**: 57, 1933; **42**: 569 and 653, 1935) and Fraser (*Surg., Gynec. & Obst.* **45**:266, 1927). This concept recognizes that the epithelium of the breast is subject to some hyperplastic stimulus which may produce local or widespread benign neoplasia of various types. Papillary neoplasia, at first benign, may progress to carcinoma from one or multiple foci of origin, from duct rather than acinar epithelium. This process parallels the development of carcinoma from multiple polyposis of the colon or stomach. While this appears to be the most frequent mode of histogenesis, primary malignant transformation of duct epithelium directly, without passing through a benign papillary stage, is also possible and may account for the finding in a single case of a tiny area of intraductal cancer without the more usual picture of a distended cystic or papillary site of origin. This theory excludes squamous and transitional cell cancer, which are clearly related to the epithelium of the nipple.

DISCUSSION

IRVING GRAEF: This study was undertaken originally in an attempt to prepare a critique of the grading of mammary carcinoma. While severe criticism of the use of grading as a prognostic aid has been offered by some investigators—Dr. Plaut himself severely criticized the procedure in a general review—no statistical and careful serial study has been published to bring out the inefficacy of using random sections to determine the growth characteristics of a tumor. Soon it was learned in Dr. Koppelman's study that a great deal more may be said about the process when the whole gland is subjected to a simple macroscopic serial study, supplemented by the use of key blocks, which are simply those which represent the tumor and surrounding tissue.

CHICAGO PATHOLOGICAL SOCIETY

KATHARINE M. HOWELL, *President*

Regular Monthly Meeting, Jan. 9, 1939

EDWIN F. HIRSCH, *Secretary*

PATHOLOGIC AND ETIOLOGIC OBSERVATIONS ON AN INTESTINAL ENDEMIC OF NURS-LINGS. ERNEST A. PRIBRAM.

An intestinal endemic among newborn infants observed in the fall of 1937 with extremely high morbidity and mortality was caused by *Aerobacter mucosum*

(Friedländer's bacterium). The micro-organisms isolated from the intestinal contents in life, as well as at necropsy, and from the lungs and heart at necropsy were highly virulent for mice. The virulence could be demonstrated by injecting, as well as by feeding, the cultures. The virulence was considerably lower and was eventually lost in strains transplanted daily and continuously on plain agar. There was a marked decrease of virulence in strains planted in the same way on dextrose and also on lactose mediums. There was, however, no loss but rather a gain of virulence in cultures grown constantly on galactose agar. Virulence was commensurate with slimy character and capsule formation. Mice fed cultures grown on plain agar or on dextrose or lactose agar gradually overcame the infection, whereas mice fed strains grown on galactose mediums had pure cultures of these bacteria in their stools; the bacteria replaced the normal intestinal flora and caused malnutrition of the infected animals. Lactose in diets of newborn infants may aggravate an intestinal infection with *A. mucosum*.

SEROUS HEPATITIS. HANS P. POPPER.

This is a report of investigations made in collaboration with Eppinger on serous inflammation. Capillaries are semipermeable tubes which retain the plasma proteins. The first manifestation of capillary damage is a loss of semipermeability with escape of proteins into the interstitial tissue. There the proteins, which are not reabsorbed by the blood capillaries and only gradually absorbed by the lymphatics, bind water and cause edema. This process, called serous inflammation, is known to occur in serous cavities and subcutaneous tissue. The significance of it in the soft interstitial tissue of the large parenchymatous organs has only recently been evaluated (Eppinger and Roessle).

Serous hepatitis is characterized by enlargement of the spaces between the liver cell cords and the blood capillaries, Disse's spaces. They become filled with protein granules, especially after fixation with a solution of glacial alcohol in absolute alcohol. In human livers serous hepatitis is often present, even after an agony of longer than usual duration, but it is especially marked in the livers of persons who died of infections, intoxications, coma or burns. In animals it can be produced by allylformiate, a representative of substances isolated from purulent exudates or spoiled meat. Serous hepatitis can be produced in many of the lower animals—for example, in the salamander. The development depends on the size of the reticulofibrous net in Disse's spaces. In man or dogs the space is large; in rabbits or guinea pigs it is small, and therefore in these animals the tendency to serous hepatitis is not marked. In acute intoxications of dogs, besides the changes of Disse's spaces, there is edema of the periportal fields and of the gallbladder bed, both having lymph vessels with an abundance of fluid. All three signs together form the triad of serous hepatitis.

To study the consequences of serous hepatitis, dogs were chronically intoxicated with allylformiate. After peroral or peritoneal introduction the effects were much severer than with subcutaneous injection. As quickly as twenty-four hours after administration of the drug there was complete destruction of the periphery of the lobules with rupture of the reticulofibrous framework. These ruptures, the severest degree of capillary damage, stimulated proliferations. Thus, finally a condition like cirrhosis developed in the course of two weeks. A cirrhosis very similar to that in man was produced in about six weeks by simultaneous use of small doses of allylformiate and bacteria intravenously. Then localized destruction of the periphery of the lobules appeared with formation of exudates and proliferation of the connective tissue. The rupture and occlusion of the communication between the bile capillaries and ducts explain the obstructive jaundice and, further, the proliferation of the smaller bile ducts, which is a result of stimulation by the rupture and attempts to reunite. In the human pathologic process the complete triad of serous hepatitis is not common. It is typically present in beriberi and in catarrhal jaundice, as far as one can judge from the clinical and postmortem observations in one case.

Serous hepatitis is, because of the specific structure of the liver, the first response of the organ to any irritation, irrespective of the cause. Further than this, it may disappear entirely or it may be followed by destruction of the parenchymatous cells or rupture of the framework built by the connective tissue (cirrhosis).

CARCINOMA OF THE PARATHYROID GLAND. KARL A. MEYER, PETER A. ROSI AND ALEX B. RAGINS.

Carcinoma of the parathyroid gland occurred in a Greek aged 56. The man had symptoms of hyperparathyroidism, such as pains in the bones and joints, a generalized fibrocystic disturbance of the bones, renal concretions, hypercalcemia, a decrease in the phosphorus of the serum and an increase in the phosphatase of the blood. Removal of the tumor ameliorated the pain in the bones and joints. Roentgenograms demonstrated definite recalcification of the fibrocystic tissues. The serum calcium and phosphorus and the blood phosphatase also reached normal levels temporarily. About one year after parathyroidectomy the pain returned, the serum calcium rose, the serum phosphorus fell and the serum phosphatase increased slightly. Roentgenograms disclosed increased fibrocystic changes of the bones. In the right side of the neck was a recurrent firm fixed mass. These late symptoms indicated persistent hyperparathyroidism.

RECOGNITION OF SUBGROUP A_2 AS A MEANS OF AVOIDING BLOOD TRANSFUSION REACTIONS. I. DAVIDSOHN.

Clinical experience, as it is expressed in the literature, suggests that the selection of a donor according to the known methods does not assure the absence of reaction to a blood transfusion, that such unexpected reactions are not uncommon when donors of the same blood group as the patients are employed, especially when donor and patient are of blood groups O and A, and that the reactions are particularly frequent when the so-called universal donors are employed.

Available serologic data suggest that subgroups A_1 and A_2 , A_1B and A_2B may not be compatible, that the subgroups A_2 and A_2B are not infrequently mistaken for other blood groups, particularly for O and B, and that some reactions to blood transfusions, even fatal, are explained by the aforementioned circumstances. A high-titered, easily produced and highly specific immune rabbit serum enables a prompt recognition of blood group A, including the feebly agglutinating subgroup A_2 . The proper dilution of the serum as determined by titration, makes possible a differentiation of subgroups A_2 from A_1 without delay. Both procedures may be completed within five minutes.

This article was published in full in *The Journal of the American Medical Association* (**112**:713, 1939).

KATHARINE M. HOWELL, *President*

Regular Monthly Meeting, Feb. 13, 1939

EDWIN F. HIRSCH, *Secretary*

IN VITRO AND IN VIVO ACTION OF CHRONIC INFLAMMATORY TISSUE ON CERTAIN ANTIGENS AND ANTIBODIES. KATHERINE E. HITE.

Bacteriologic and immunologic studies on chronic staphylococcic osteomyelitis demonstrated that some interaction occurs in the chronic lesion which markedly affects the immunologic aspects of this disease. The antihemolysin of the patient's serum, it was found, decreases during the chronic stages. In animals, the general effect of antigens and antibodies introduced into experimental fistulous tracts was significantly below that obtained when other routes of injection were used.

Typhoid vaccine introduced into the sinus tracts of patients failed to cause an increase in the agglutinin titer of the serum.

The present work has been concerned with the effect of pus and emulsions of fistulous tracts in rabbits on staphylococcus toxin, botulinus antitoxin and horse plasma in vitro, and the persistence of horse plasma in experimental sinus tracts. The presence of the test substance after the aforementioned treatment was determined as follows: staphylococcus toxin, by the hemolysin reaction with rabbit erythrocytes; botulinus antitoxin, by the mouse protection test against known toxin; horse plasma, by the reaction of anaphylaxis in sensitized guinea pigs. The results demonstrated that staphylococcus toxin, botulinus antitoxin and horse plasma were not destroyed by in vitro incubation for sixteen to twenty hours with pus and emulsions of sinus tracts and that sufficient horse plasma remained in sinus tracts for twenty-four and forty-eight hours to be detectable by anaphylaxis. These experiments tend to show that the effect of chronic inflammatory tissues in reducing the response of the host to antigens and antibodies contained within them is not essentially that of destruction of these substances but rather that the inflammatory tissue is an impermeable barrier to them.

EXPERIMENTAL CRYPTOCOCCIC INFECTION. L. R. KUHN.

Benham's contention that the Cryptococci causing meningitis and European blastomycosis cannot be readily differentiated was supported when a strain from a patient with only a subcutaneous lesion and bone involvement could not be distinguished from strains isolated from patients with meningitis.

The course of experimental cryptococcic infection in mice was studied. The infection may be detected easily by examining gram-stained preparations of tissues, particularly of the brain and lungs. Eight strains of *Cryptococcus hominis* could not be differentiated in their pathogenicity for mice. A ninth strain, however, consistently required more time to kill mice, and with this strain cysts in the brain frequently enlarged to such an extent that the parietal bones of the skull were elevated. The mice lived from one to three weeks after this became noticeable. This strain was morphologically, culturally and serologically like the others, and it appeared to multiply as rapidly in the mouse. Its pathogenicity and the pathogenicity of one of the other strains were not increased after fifteen consecutive passages in mice.

Unlike bacteria and other yeasts when injected into the peritoneal cavities of mice, each of 13 strains of cryptococci injected in quantities of 4,000,000 to 8,000,000 organisms in 0.1 cc. of 0.85 per cent solution of sodium chloride caused no greater exudation of neutrophilic leukocytes than did 0.1 cc. of saline solution. Heating, shaking with glass beads for one hour and the culture medium did not affect the in vivo chemotactic properties of the yeast for polymorphonuclear leukocytes. Cultures three to four weeks old attracted a few more of these cells; large numbers (25,000,000) of cryptococci attracted many more. Peritoneal exudates from 3 rabbits showed a similar deficient response to cryptococci.

SOME PHYSICAL AND CHEMICAL PROPERTIES OF STAPHYLOCOCCUS ENTEROTOXIN. ELLEN DAVISON.

Methods devised for the assay of staphylococcus enterotoxin are unsatisfactory. Biochemical properties and growth on differential mediums are not reliable criteria for the identification of enterotoxic strains. Oral administration and injection of the enterotoxin into monkeys and kittens have been the only methods yielding consistent results, but these methods are unsatisfactory for routine or quantitative assay. Other animals, the dog excepted, do not react. A better method of assay and an explanation for the peculiarities of this exotoxin may develop from studies of its physical and chemical properties.

Enterotoxin is antigenic. It is not destroyed by heat or formaldehyde. My studies of monkeys have demonstrated that the enterotoxic potency is diminished by heating. These animals were given intravenous injections of filtrates boiled for twenty, thirty or sixty minutes or autoclaved. Although the number of mon-

keys used for the 75 injections does not permit a definite conclusion, the trend is unmistakable. Attempts to purify the enterotoxin by precipitation with alcohol were unsuccessful. The enterotoxin was found resistant to alcohol that destroyed the staphylococcus lethal toxin. Chloroform and ether extracts of filtrates when fed to monkeys and injected into kittens had no effects. Enterotoxin was in the residue filtrate after the extraction. Enterotoxic filtrates were treated with ammonium sulfate at half and at complete saturation. None of the monkeys fed the material precipitated at half-saturation reacted, while 10 of 14 receiving that obtained by full saturation vomited.

EXPERIMENTAL STUDIES ON THE ADSORPTION OF BOTULINUM TOXIN BY KAOLIN-ALUMINUM HYDROXIDE IN THE INTESTINES OF ANIMALS. JAMES L. WATERS, G. M. DACK and L. R. DRAGSTEDT.

Guinea pigs were fed kaolin-aluminum hydroxide daily for seven days and on the eighth day were given fatal doses of botulinus toxin. In other experiments guinea pigs were fed toxin mixed with kaolin-aluminum hydroxide or were fed the supernatant fluid from such mixtures. Monkeys were fed toxin and kaolin-aluminum hydroxide mixtures and were given enemas consisting of the same mixture or untreated botulinus toxin. In vitro experiments to determine the effect of the gastric and pancreatic secretions of dogs on toxin adsorbed by kaolin-aluminum hydroxide were also performed.

Guinea pigs were not protected from botulinus toxin by the kaolin-aluminum hydroxide. Monkeys fed such mixtures survived. Only one monkey in the entire series had symptoms, and this one recovered. No symptoms appeared in monkeys which received toxin and kaolin-aluminum hydroxide enemas or untreated toxin enemas. There is evidence that the gastric secretions but not the pancreatic elute adsorbed toxin. The experiments also demonstrated that botulinus toxin is quantitatively adsorbed by kaolin-aluminum hydroxide.

DISCUSSION

G. M. DACK: The results indicate that there is no real basis for the use of kaolin-aluminum hydroxide in preventing the absorption of a toxic substance from the intestine.

NEW ENGLAND PATHOLOGICAL SOCIETY

CHARLES BRANCH, *President*

Regular Meeting, Jan. 19, 1939

GRANVILLE A. BENNETT, *Secretary*

PREINVASIVE CARCINOMA OF THE CERVIX. PAUL A. YOUNGE (by invitation).

At the Free Hospital for Women, Brookline, Mass., a group of 49 cases of preinvasive carcinoma of the cervix has been collected since Smith and Pemberton published their report of 16 cases (*Surg., Gynec. & Obst.* 59: 1, 1934). In 5 of their 16 cases the diagnosis of carcinoma of the cervix was not made on the original biopsy slides until three and a half to twelve and a half years later, when the patients began to show invading carcinoma. In spite of the malignant outcome in these 5 cases, the consensus of most pathologists disagrees with the diagnosis of carcinoma on the original biopsy slides.

Since 1934, 2 more cases have been discovered in which invading carcinoma developed two and one-sixth and three and one-third years, respectively, after the original biopsies, in which the lesions were diagnosed as benign though now they are classified as preinvasive carcinoma.

Representative slides from both of these series have been shown to pathologists, who usually made the diagnosis of "chronic inflammation," "repair process" or "precancer." Because of the disagreement, one lesion was followed for one year without treatment. Three biopsies were made during the year, at the end of which time the lesion was 4 mm. in diameter and definitely invasive.

The morphologic character of the malignant, yet noninvasive epithelium is so characteristic and consistent that frequently a cursory low power microscopic inspection reveals its nature. It is the same type of epithelium as is sometimes found at the edge of an invading cancer of the cervix. At this point there is usually a sharp oblique line of demarcation, and the malignant epithelium extends along the surface for a short distance before it shows its invasive tendency. In preinvasive cancer the morphologic and cytologic picture is the same as is frequently seen in the marginal zone of a frank cancer, namely, incomplete differentiation, atypical cells and mitoses, variation in the size of the nuclei, large and often multiple nucleoli, occasionally multinucleated cells, hyperchromatic nuclei, and mitoses in the upper layers of the epithelium instead of in just the basal layer. The actual number of mitoses need not be increased over that of healing epithelium.

(Dr. Younge showed a representative group of photomicrographs in illustration of the 65 cases, including the case in which there had been no treatment, together with a few color photographs of the extirpated cervixes demonstrating the gross pathologic appearance and results of the Schiller test. Several biopsy specimens from lesions which later were proved to be frank cancer, not included in this series, were shown to demonstrate the difficulties of making a correct diagnosis from one section. A group of slides showing the abnormal conditions, ranging from the most controversial to the more obviously malignant, were examined by several pathologists, and their diagnoses were tabulated and discussed.)

The conclusion from this study is that when biopsy of a specimen of the cervix shows preinvasive carcinoma, or carcinoma in situ, repeated biopsies will reveal either the same condition or actually invasive carcinoma.

DISCUSSION

SHIELDS WARREN: This is a most interesting and important presentation. Several years ago, when I first had the privilege of seeing some of these slides, I was much more conservative in my interpretation of them than I am at the present time. It is a serious question as to where pathologists should draw the line of actual malignancy. They used to think that without evidence of invasion there could be no malignancy. While it is difficult to give up this point of view, one must give very serious consideration to the conception of carcinoma in situ. Much as I have disliked the term, I feel that it probably is too useful to be discriminated against. Pathologists must be alert to recognize the changes so clearly pointed out by Dr. Younge and to follow them carefully in order to determine what happens to them.

Although a lesion such as was observed in these cases, showing atypical hyperplasia and numerous mitotic figures without invasion, must be considered malignant in the light of the experience presented, I believe that it should be more conservatively treated than a fully developed carcinoma. Pathologists certainly must urge biopsy of every suspicious lesion and have carcinoma of the cervix in mind from the onset of the child-bearing period. The comparatively youthful age of many patients with carcinoma of the cervix must be realized.

TRACY B. MALLORY: No one, I am convinced, can study the remarkable material which Dr. Younge has just presented and not be convinced both of the feasibility and of the importance of recognizing carcinoma in situ. It must be admitted that the gynecologists of the Free Hospital for Women have taught pathologists a most salutary lesson in their own field of histologic diagnosis. In retrospect it seems surprising that the pathologists in Boston should have been so slow in accepting the diagnosis of carcinoma in situ, since what was probably the first good histologic description of such a lesion was Dr. Bowen's description

of precancerous dermatitis, a lesion which has been known ever since as Bowen's disease. Perhaps, in fact, that has been part of the trouble. Pathologists had a mental image of carcinoma in situ derived from a study of cutaneous lesions and presumed that cervical lesions would present the same appearance.

A prophet is without honor in his own country. It seems only fair at this moment to point out that Dr. Frank Pemberton and Dr. George Van S. Smith began to talk about noninvasive cancer a full ten years ago. They sent slide after slide to various pathologists, who almost unanimously refused to make a diagnosis of malignant growth because there was no invasion. The end results which Dr. Younge has presented can leave no question as to who was right. It was not until Prof. Walter Schiller, of Vienna, came to Boston three years ago and lectured before this society on noninvasive cancer that the members granted to the foreign prophet the credence they refused to the local ones. Since that time, although conservative instincts still make them hesitate, I think that few of them have failed to diagnose carcinoma in situ on some occasion at least.

It must be admitted that the group at the Free Hospital for Women are exceptionally well fitted for such an investigation as this. The fact that the men who study the patients clinically also diagnose the conditions presented on the slides makes for a much closer correlation of clinical and pathologic observations than is possible in the average general hospital. Moreover, the final and most convincing stage of their demonstration, the repeated observation of a carcinoma in situ until it finally becomes invasive, is an unjustifiable experiment except in the hands of a clinician so sure of his control of the patient that he is confident the experiment can be interrupted before the patient's chances of cure have been jeopardized.

E. P. MCCARTHY: I have been interested in leukoplakia of the oral cavity, a lesion which runs somewhat parallel to lesions of the type presented by Dr. Younge.

Leukoplakia in the oral cavity rarely begins before the age of 40 and is often the result of a known chronic irritation. Such lesions often become verrucous. I should like to ask Dr. Younge whether or not lesions of the type he has described ever become verrucous, and what in his opinion is their cause.

GEORGE VAN S. SMITH (by invitation): We at the Free Hospital for Women have enjoyed not only giving our opinions concerning sections of the cervix that presented unusual and suspicious changes and then checking back to learn the clinical outcome but also studying previous biopsy specimens, whenever possible, from patients with obvious cervical cancer. I think it very likely that such minor procedures as trachelorrhaphy and cauterization may eliminate an occasional early cancer. For example, recently in reviewing sections prepared before 1910 we found two which appeared to us to contain malignant epithelium. Both were from specimens obtained at trachelorrhaphy. One of the patients died of carcinoma of the stomach (diagnosed at the Massachusetts General Hospital) over six years after trachelorrhaphy; the other died of alleged carcinoma of the endometrium over twenty years after trachelorrhaphy. Either these patients did not have pre-invasive cancer or were cured by their plastic operations. Biopsy of a third patient's cervix in 1934 revealed what we considered one of these early malignant lesions—and our diagnosis was confirmed by a pathologist in Boston. It was decided to follow the patient carefully to determine future developments. At the time of the second biopsy, one month after the first, the cervix was thoroughly cauterized. I have been examining this patient periodically for four and one-half years—she is now well, with no evidence of cervical disease. Cauterization probably destroyed an incipient cancer. I think this answers Dr. Warren's question about "pulling our punches" on these early cancers, but we emphatically do not recommend these procedures as treatment for any cervix with indisputable cancer, no matter how early it is. Further, as Dr. Tracy Mallory emphasized, these procedures should not be relied on, even in patients with debatable lesions, unless they can be followed with certainty and examined often.

With the increase in biopsy material at the Free Hospital for Women and consequently the more frequent finding of these early malignant processes, we often have the disturbing thought that we must be missing some early cancers and that others must be missing a few. For example, last fall we treated a frank cancer of the cervix in a patient who had had a supravaginal hysterectomy for pelvic inflammation at our clinic three years previously, at which time the cervix was not considered sufficiently abnormal to "rate" biopsy or treatment. We probably missed a very early lesion in this patient.

PAUL A. YOUNGE: The treatment of preinvasive carcinoma of the cervix, or carcinoma in situ, should be exactly the same as for invading carcinoma, because frequently the first biopsy specimen shows only preinvasive carcinoma, whereas larger secondary specimens show definite invasion. This fact was emphasized during the presentation of the photomicrographs. If conservative treatment is desired, high amputation of the cervix may be done. If, however, careful pathologic examination of the extirpated cervix reveals invasion, radium and roentgen treatments should be given immediately, or as soon as the wound heals. Another method is complete hysterectomy, but, again, as with amputation of the cervix, if the lesion is found to be invasive, radium and roentgen treatments should be given.

I am glad Dr. Tracy Mallory brought out the fact that Dr. Frank Pemberton and Dr. George Van S. Smith began talking about this subject over ten years ago. Even before 1927 they began diagnosing these lesions as carcinoma, but general pathologists would not agree with them. However, in most cases they treated the patient for carcinoma in spite of the disagreement as to the diagnosis.

We do not associate kraurosis or verrucose-like lesions of the vulva with carcinoma of the cervix. Leukoplakia of the cervix has been reported a few times to develop into carcinoma, but this is open to question. Schiller does not believe that leukokeratoses develop into cancer. We have not followed any long enough to have an opinion. He also does not believe chronic inflammation is an etiologic factor. I feel that chronic inflammation may play a part in the development of carcinoma of the cervix, but the most important thing is to make routine biopsies of tissue from the junction of the normal squamous epithelium and the eversion, erosion or ectropion, as well as of all positive Schiller spots. Annual inspection of the cervix, with biopsy if indicated, in all women over 30 and in women of any age if they have borne children should be the established practice. This plus treatment of all eversions, erosions or ectropions will reduce the incidence and mortality of the disease.

GEORGE VAN S. SMITH: Dr. Younge's reply to Dr. McCarthy's question as to what may be a predisposing factor in the causation of cervical cancer demands, I believe, modification. In general, opinion is falling away from the idea that chronic inflammation may be etiologic. Knowing that the incidence of cancer of the cervix is greater in women who have been pregnant than in nulligravidas and that pregnancy is associated with a tremendous increase in estrogens and that these substances not only play a part in the induction of certain experimental cancers but also are closely related chemically to carcinogenic agents, I wonder whether these physiologic sterols may not act like, or even become, carcinogenic sterols in the patient susceptible to cancer.

Book Reviews

Parasitology, with Special Reference to Man and Domesticated Animals.

Robert Hegner, Ph.D., Professor of Protozoology, Johns Hopkins University; Francis M. Root, Ph.D., Late Associate Professor of Medical Entomology, Johns Hopkins University; Donald L. Augustine, Sc.D., Assistant Professor of Helminthology, Harvard University, and Clay G. Huff, Sc.D., Associate Professor of Parasitology, University of Chicago. Cloth. Pp. 812, with 308 illustrations. Price, \$7. New York: D. Appleton-Century Company, Inc., 1938.

This edition of the authors' well known textbook entitled "Animal Parasitology," follows the same plan as the original edition. The introduction, by Hegner, gives a survey of such general aspects of parasitism as animal habitats, the types of host-parasite relationship, the occurrence, origin, evolution and effects of parasitism and the rules of zoologic nomenclature. This is followed by considerations of the three natural divisions of parasitology: protozoology (Hegner), helminthology (Augustine) and present knowledge of those arthropods which are of parasitologic importance. The latter section was originally written by Root and has now been revised by Huff. In addition to these sections there is a bibliography covering 79 pages.

In general the conception and execution of the text are excellent. It stresses various fundamental biologic concepts in the field of parasitology and at the same time gives up-to-date information on various pertinent details of medicine, public health and technic. Criticisms regarding the completeness of various sections are largely matters of individual judgment, as no general textbook can aspire to completeness. Thus, it seems to this reviewer that certain aspects of important infections should have been considered in greater detail. The sections on the pathologic aspects of malaria and on immunity to this disease, for example, are very incomplete and contain misleading statements. Splenomegaly in malaria is ascribed "to distention with blood as a result of lowered vascular tone" without consideration of the additional factor of hyperplasia of various cellular elements after long-continued malarial stimulation. In the section on immunity the statement "Apparently the destruction of parasites within the host is due largely to the phagocytes, known as macrophages, that live on the walls of the blood vessels of the internal organs" is not very enlightening as to the origin of macrophages and will be interpreted by many as indicating a phagocytic nature of the common vascular endothelium of all internal organs. In spite of these minor shortcomings, the book will undoubtedly continue to hold its place as one of the outstanding texts on parasitology in English.

Cancer: Its Diagnosis and Treatment. Max Cutler, M.D., Associate in Surgery, Northwestern University Medical School, Chicago, and Franz Buschke, M.D., Assistant Roentgenologist, Chicago Tumor Institute, Chicago. Assisted by Simeon T. Cantril, M.D., Director, Tumor Institute, Swedish Hospital, Seattle. Cloth. Pp. 757, with 346 illustrations. Price \$10. Philadelphia: W. B. Saunders Company, 1938.

The purpose of this book is to promote the application of the knowledge and methods at hand to the early diagnosis and treatment of cancer. Throughout the book the word "cancer" includes malignant tumors in general. The book opens with a comprehensive chapter on radiotherapy, in which are discussed such topics as the biologic effects of roentgen rays and of the gamma rays of radium, the methods of radiotherapy and the radiosensitivity of cancers. This chapter and the next two, on biopsy and on the spread of cancer, are of special interest to the

pathologist. The rest of the book is devoted to the systematic, orderly consideration of the diagnosis, the treatment and the results of treatment of cancer in all parts of the body except the eye and the central nervous system. There are 346 illustrations, all highly instructive. They show gross appearances of tumors in situ and otherwise, roentgenograms, anatomic relations of importance in the metastasis and in the treatment of cancers, appliances and methods used in radiotherapy, and the typical microscopic structure of important cancers. Minute details of morphologic appearance and histogenesis are left out of consideration purposely. Undoubtedly the lack of illustrations in the case of the thyroid, the urinary bladder, the testicle and the prostate will be supplied in future editions. The bibliography is consolidated conveniently at the end by chapters and alphabetically according to the names of the authors cited in the text. There is also a name index and a good, complete subject index. The book is an important addition to the literature on cancer in its clinical aspects. A particularly strong point is the presentation of the advances of radiotherapy of cancer. There is no recent book on clinical cancer in which the microscopic aspects of cancer are presented better on the basis of first hand knowledge. The pathologist will find here a thoroughly competent discussion of the structure and course of cancer in close correlation with the details of clinical diagnosis and treatment under different conditions.

Laboratory Manual of Hematologic Technic. Regena Cook Beck, M.A., M.D., Formerly Instructor in Pathology and Bacteriology at George Washington University Medical School; Head of the Department of Bacteriology, William and Mary College Extension; Pathologist to Stuart Circle Hospital and Director of the Stuart Circle Hospital School of Medical Technology, Richmond, Va. With a Foreword by Frank W. Konzelmann, M.D., Professor of Clinical Pathology, Temple University, Philadelphia. Cloth. Pp. 389, with 79 illustrations. Price \$4. Philadelphia and London: W. B. Saunders Company, 1938.

This manual describes and explains in admirable fashion the various procedures of modern hematologic technic. Also, it interprets the results and their significance. There are five parts, dealing with the following topics: the methods of procuring blood specimens, the mechanism of clotting, the estimation of the hemoglobin content; the enumeration of blood cells and the determination of indexes; the cytology of blood; special studies used in hematologic practice; the special pathology of blood. The summaries, the lists of questions and the definitions of terms will be of great help to the student. The presentation is competent, comprehensive and clear. The book is well described in the following statement from the foreword by F. W. Konzelmann: "This manual is a valuable guide not only for the technologist, but all students of hematology will find it most helpful, whether they be pathologists, clinicians or medical students, for there is at the time of this writing no other single volume where so much information on the subject of procedure in hematology is contained between two covers." The book should have a place in every clinical laboratory.

Classic Descriptions of Disease with Biographical Sketches of the Authors. Ralph H. Major, M.D., Professor of Medicine, University of Kansas School of Medicine, Kansas City. Second edition. Cloth. Pp. 727, with 137 illustrations. Price \$5.50. Springfield, Ill.: Charles C. Thomas, Publisher, 1939.

The first edition of this very interesting book was published in 1932. In the new edition sections have been added on malaria and yellow fever; also, additional readings and illustrations. Many of the biographic sketches have been rewritten, and the index has been revised. The book contains a great wealth of readings and information to illustrate the development of knowledge of disease.

Books Received

THIRTY-SIXTH ANNUAL REPORT 1937-1938 OF THE IMPERIAL CANCER RESEARCH FUND. Under the Direction of the Royal College of Physicians of London and the Royal College of Surgeons of England. Paper. Pp. 39. London: Royal College of Surgeons, 1938.

THE PASTEUR INSTITUTE OF SOUTHERN INDIA, COONOR. The Annual Report of the Director for the Year ending 31st December 1937 Together with the Thirty-First Annual Report of the Central Committee of the Association for the Year Ending 31st March 1938. Paper. Pp. 89. Madras: The Madras Publishing House, Ltd., 1938.

MEDDELELSER FRA DR. F. G. GADES PATHOLOGISK-ANATOMISKE LABORATORIUM I BERGEN, 1938. Paper. Various pagination. 1939.

LA PONCTION STERNALE. PROCÉDÉ DE DIAGNOSTIC CYTOLOGIQUE. P. Émile Weil, Médecin des Hôpitaux de Paris, and Suzanne Perles, Chef de laboratoire a l'Hôpital Tanten. Paper. Pp. 184, with 25 illustrations. Price 75 francs. Paris: Masson & Cie, 1939.

WILLIAM P. WHERRY, BACTERIOLOGIST. Martin Fischer. Cloth. Pp. 293. Price \$4. Springfield, Ill.: Charles C. Thomas, Publisher, 1938.

LEHRBUCH DER ALLGEMEINEN PATHOLOGIE UND DER PATHOLOGISCHEN ANATOMIE. H. Ribbert. Twelfth edition. Edited by Prof. Dr. H. Hamperl, Prosektor am pathologischen Institut der Universität, Berlin. Paper. Pp. 634, with 700 illustrations. Price 27 reichmarks. Berlin: F. C. W. Vogel, 1939.

BASE-PROTEIN-ACID COMPOUNDS PREPARED FROM FIBRIN

MARTIN H. FISCHER, M.D., Sc.D.

AND

WERNER J. SUER, M.S.

CINCINNATI

I

These pages extend remarks made earlier¹ wherein biologic and chemical evidence was cited which proves protoplasm (including blood, lymph, milk and egg white) not a mere mixture of protein with salt and water. The physical and chemical characteristics of living matter are repeated only when a chemical unit is made of the three. Thereafter it was shown, in the instance of casein, how such hydratable base-protein-acid compounds might be prepared. What is requisite is that the necessary chemical reactions be permitted to proceed as under the conditions existent in protoplasm, wherein the average water content of 80 per cent is all held in combined form. In other words, no "free" water may be present in the reaction mixture. The following paragraphs show how such triple compounds may be prepared from the fibrin of blood.

"Pure" fibrin absorbs little water (its capacity to "swell" is low). Enormous increase occurs if either an alkali or an acid is added, because the resultant proteinates have great hydration values.² Neither of these compounds alone, however, may be taken as the chemical analogue of fibrin as it exists in nature, where both acid and alkali appear in it (as the commonly designated mixture of "salts" ashable out of it), in the approximate relation of 0.7 of the former to 1 of the latter.

The "pure" fibrin employed in the experiments described here was obtained by first bringing a commercial fibrin into "solution" (really into a state of hydration miscible with more water) in strong potassium hydroxide. After this mixture had been filtered, the fibrin was reprecipitated through neutralization with strong hydrochloric acid. After

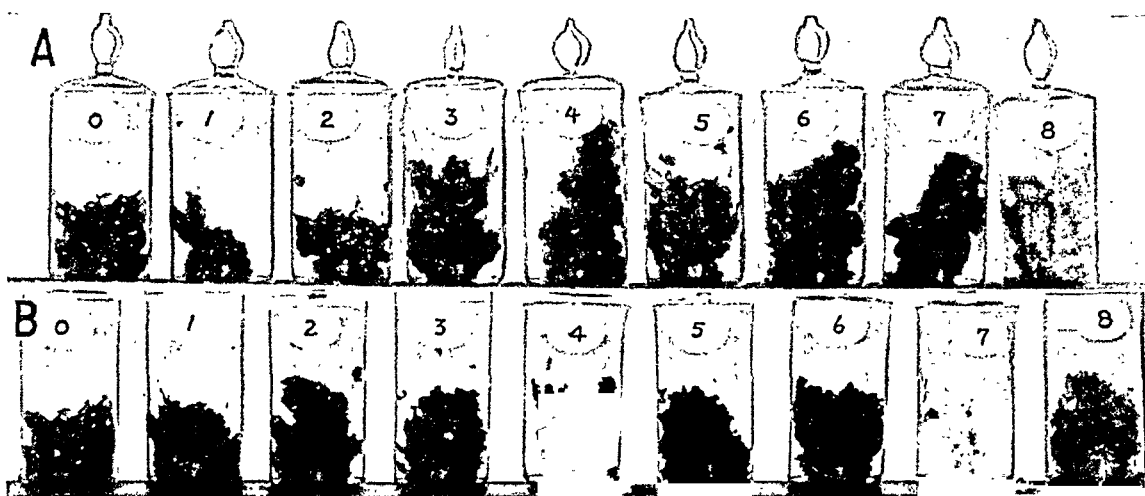
From the Laboratory of Physiology, University of Cincinnati.

1. Fischer, M. H., and Suer, W. J.: *Arch. Path.* **20**:683, 1935.

2. Fischer, M. H.: *Oedema and Nephritis: A Critical, Experimental and Clinical Study of the Physiology and Pathology of Water Absorption by the Living Organism*, ed. 3, New York, John Wiley & Sons, Inc., 1921, p. 61.

several washings with water it was covered with 95 per cent alcohol and stored under it.

Example: One hundred grams of powdered fibrin was allowed to swell for twenty-four hours in the ice box in 1,000 cc. of water, after which 67.5 Gm. of pure potassium hydroxide dissolved in 500 cc. of water was added. The half-gelatinous mixture was permitted to stand twenty-four hours, and then, to render the mixture more liquid, 500 cc. of water was added and the whole filtered. Concentrated hydrochloric acid was then slowly stirred into the filtrate until maximal precipitation was obtained. When 81.1 cc. had been added, the supernatant liquid was faintly acid to litmus. It was decanted, and the precipitated fibrin was washed throughout a day with several changes of water, after which the moist precipitate was covered with three volumes of 95 per cent alcohol. After standing another day, the precipitate was filtered off, washed with more alcohol and preserved under it. Such fibrin was still highly solvated. From the alcohol-wet yield of 126 Gm. in the example cited, an aliquot portion showed 33.14 Gm. to be



A shows how hydrochloric acid in increasing quanta may be added to the 75 per cent hydrated potassium fibrinate jelly shown in 0 without visible effect on its physical characteristics. *B* illustrates the same observation when a definite quantity of each of a series of different acids was added to the standard potassium fibrinate gel shown in 0.

solid (26.3 per cent). Nor is it to be considered absolutely pure, for on ashing we still recovered 0.365 per cent potassium chloride (which, however, was not merely adherent to but an intimate part of the protein).

To prepare potassium fibrinate, we separated the 126 Gm. of solvated fibrin, obtained as described, from its surrounding fluid by filtration. In the process the wet weight shrank to 102 Gm. To the resultant, 25 cc. of water and 13 cc. of twice normal potassium hydroxide were added. After twenty-four hours the clear solid jelly shown in tube 0 of *A* in the figure was obtained. Counting in any alcohol remaining, one may say that this product did not carry more than 75 per cent of water (not more, therefore, than the physiologic maximum of tissue).

The remaining tubes of *A* show how *strong hydrochloric acid may be added to this potassium fibrinate gel without change in its physical*

character. The acid does not strike the base off the proteinate but adds itself to the protein when, as here, free water is absent. Not until tube 8 is reached, in which the amount of acid added exceeds 70 per cent of the base present in the proteinate, does combination with the base occur and precipitation of fibrin in a "neutral" and less hydrated form come about (the gel turns white).

B illustrates the production of such "triple" compounds from several different acids. The control potassium fibrinate gel with 75 per cent water is again shown under 0. In the remaining tubes, tartaric, hydrobromic, citric, acetic, lactic, hydrochloric, phosphoric and sulfuric acids have been added in this order in chemically equivalent amounts up to 69 per cent of the alkali contained in the original compound. The colloid character of the gel was in no wise altered except in the last two tubes (to understand this it needs to be recalled that protein phosphate and protein sulfate stand lowest in *absolute* hydration value in any protein series so far studied).

II

To be brief, we condense some further observations on the fibrin compounds described here (and the caseinates noted earlier) into the following paragraphs.

The alkali proteinates or the acid proteinates of either take up more water ("swell more") than the proteins alone. *Their hydration capacities* are thus increased. But specific differences appear as different bases or different acids are employed. The absolute hydration values are greatest with potassium, ammonium and sodium, much lower with the earth metals and lowest with the heavy metals. In an acid series, the compounds with hydrochloric, hydrobromic and lactic acid stand at the top, the compounds with various organic acids lie lower and the compounds with phosphoric and sulfuric acids bring up the bottom. Thus the so-called "physiologic" degree of hydration characteristic of living matter (its normal water content) may be assigned to the mixture and the relative proportions of the various bases and acids that appear in it (the relative quantities and qualities of its "salts"); and the hydrating or dehydrating effects of pharmacologically applied bases, acids or salts, to the additive or substitution effects which the constituent radicals have on the substrate's first composition. So while all alkalis and acids increase the swelling of a protein not previously saturated (this is the case, too, for all "normal" protoplasm), all salts (with the exception of ammonium and possibly potassium) tend uniformly to bring about dehydration. But at the same "osmotic" concentration, their relative effectiveness is very different, the sequence of the different alkali or acid radicals being that given.

The different alkali proteinates show, too, a *different miscibility with water* (as ordinarily put, a different "solubility" in water). Only the

light metal proteinates mix easily with more. And this they do as does egg white or blood plasma or tissue juice, in all of which (as "neutral" to indicators as the alkali proteinates described here when their hydration capacity has not been exceeded) pronounced "alkalinity" develops (due to hydrolysis) when more water is added. This miscibility continues if a "physiologic salt solution" (eighth to sixth molar sodium chloride) is employed instead of water, but the development of end "alkalinity" is then much reduced. The acid proteinates behave like the alkali compounds; only, when they are mixed with water, the end reaction is acid instead of alkaline.³

What is the behavior of base-protein-acid compounds under these descriptive heads? *The introduction of increasing increments of acid into an alkali proteinate reduces progressively both its capacity to swell and its miscibility with water or salt solution.* A protein saturated with both base and acid (as the potassium-fibrin-chloride shown in tube 7 of *A*) fails to mix with water or a salt solution as completely as a beef-steak. But the base-protein-acid compounds also all take up a defined quantum of water. If not completely "saturated" with either alkali or acid, they "swell" when more of either is added (as do living cells when made edematous by such treatment). In neutral salt solutions the triple compounds shrink (are dehydrated) as the concentration of any nonreactive salt about them is increased. Herein their behavior parallels the so-called "osmotic" behavior of individual cells and tissues toward similar "hypertonic" solutions. But if the added salt is of the heavy metal variety or is possessed of a greatly dehydrating acid radical, all osmotic rules are off and the low hydration capacity of the ultimately formed compound dominates the picture. (These are the effects of those "salts" which have always proved "exceptional" in their osmotic behavior when tested on living cells.)

Regarded collectively, therefore, the properties of these base-protein-acid compounds are such that they register large resistance (meaning no visible change in physical properties) to rather large change in surroundings. They are "buffered" against the action of water, of various salts and of acids and alkalis even as are living cells. In biologic terms, their "factor of safety" is large against "injury."

SUMMARY

Base-protein-acid compounds have been produced from fibrin. Their "behavior" toward water, salts, acids and alkalis is described. It parallels qualitatively and quantitatively the reaction of living cells to like changes in surroundings.

3. How this explains the origin of acid or alkaline secretions from neutral secretory glands (salivary, pancreatic, gastric, sudorific or renal) has been touched on before (Fischer, M. H.: *Soaps and Proteins: Their Colloid Chemistry in Theory and Practice*, New York, John Wiley & Sons, Inc., 1921, p. 78).

FIBRIN AS CATALASE

MARTIN H. FISCHER, M.D., Sc.D.

AND

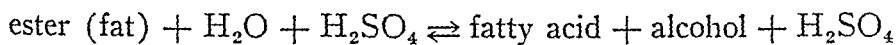
WERNER J. SUER, M.S.

CINCINNATI

I

It has been shown in previously published articles that various proteins extractable from protoplasm yield gelatinous systems, through treatment with alkalis, acids¹ or both together,² which display a varying hydratability, high electrical resistance, great viscosity and optical behavior identical with the similar properties of living matter. More recently a report on their solvent characteristics was added.³ It was shown that protein hydrates "dissolve" various materials less soluble in equal volumes of water or insoluble in it. This added proof that protoplasm is free from ordinary water was then used to explain why so many dehydrolyses (syntheses) occur in it which in the alimentary tract (where "free" water is present) appear only as hydrolyses (analyses or "digestions").

A number of chemical set-ups that fortify the rule were described. The following is picked for illustration:



This much studied reaction proceeds left or right (with synthesis or analysis *complete!*) depending solely on the absence or the presence of free water in the total system. Consideration of the equation reveals these facts: In the synthetic half the sulfuric acid acts not only (*a*) to bind any water liberated in the reaction (thus keeping the total system anhydrous) but (*b*) as its "catalyst."

Applied to living matter (or to the chemical fractions extractable from it), this generalization makes evident that any of its various "hydrophilic colloids" (all its proteins, all its carbohydrates and even some of its "peculiar" fats, therefore) act as does the sulfuric acid in the foregoing equation. Thus any or all of them may be considered

From the Laboratory of Physiology, University of Cincinnati.

1. Fischer, M. H.: Oedema and Nephritis: A Critical, Experimental and Clinical Study of the Physiology and Pathology of Water Absorption by the Living Organism, ed. 3, New York, John Wiley & Sons, 1921, p. 151. Fischer, M. H., and Hooker, M. O.: The Lyophilic Colloids, Springfield, Ill., Charles C. Thomas, Publisher, 1933, p. 230.

2. Fischer, M. H., and Suer, W. J.: Arch. Path. **20**:683, 1935.

3. Fischer, M. H., and Suer, W. J.: Arch. Path. **26**:51, 1938.

active in the establishment of the proper milieu for the activity of any ferment of the "dehydrolase"⁴ type. These paragraphs emphasize that in addition *they may be the "enzyme."* Thus we try to make "catalysis" as the name of an *activity* into the name of a *thing*; we attempt to redefine "protoplasm," heretofore regarded merely as the *seat* of ferment activity, as the *ferment itself*. Universality of distribution and overweight in tissue analysis fix a primary interest on the *proteins* of living matter.

II

The capacity of lymph, blood or tissue cells to decompose hydrogen peroxide is historic. Thenard in 1818 described the effects of metals, oxides and "fibrin" on the reaction. Besides its practical employment for decades past (as in the treatment of wounds), the reaction has come to underlie every theory of "catalysis" (Berzelius, 1835) and of "fermentation" (Schönbein, 1863) proposed since.⁵

The reaction $\text{H}_2\text{O}_2 = \text{H}_2\text{O} + \text{O}$ is accelerated by an almost universally present extractive of tissue to which the name "catalase" has been assigned. It is active in minimal concentration, is recoverable from the end products of the reaction, brings about an "unlimited" amount of decomposition and is destroyed by heat, various "poisons" and other agents, wherefore it has long been assigned to that group of catalysts of protoplasmic origin accepted today as the "ferments." The next paragraphs tend to show that a simple protein (specifically the fibrin of blood) under biologic circumstances behaves in every way like catalase.

III

The fibrin clot whipped out of spontaneously coagulating bovine blood, well washed in water and dried, and presenting thus the ordinary "commercial" product of the chemists' shelves, does not, when added to a dilute hydrogen peroxide mixture, decompose it. As some might put the matter, it is without catalase activity and "dead." The material is not easily brought back into "solution" (really, into a state of hydration miscible with water). In most acids or alkalis it merely swells.⁶ But when it is soaked in concentrated potassium hydroxide (less obviously in ammonium hydroxide) for a day or two in the ice box and stirred, a thick slime of uniform composition is obtained which can be diluted with more water and filtered through a coarse filter. The "dissolved" fibrin may be precipitated again by neutralization with hydrochloric acid. By this method we⁷ "purified" (with great loss,

4. Carl Oppenheimer's term.

5. The story is best recited in Wilhelm Ostwald's Nobel-laureated thirty-two page monograph entitled "Ueber Katalyse" (Leipzig, S. Hirzel, 1902).

6. Fischer, M. H., and Moore, G.: Am. J. Physiol. **20**:330, 1907.

7. Fischer, M. H., and Suer, W. J.: Arch. Path., this issue, p. 811.

because of "digestion"?) a commercial fibrin (already of the rather low ash content of 0.709 per cent) to serve as the stock for the experiments now to be described.

The history of one of the several batches utilized in the experiments to be described is as follows: Two hundred grams of dry fibrin was powdered and allowed to swell twenty-four hours in the ice box in 2,000 cc. of water, whereafter 135 Gm. of pure potassium hydroxide dissolved in a liter of water was added to the gelatinous mixture. After two days more of soaking in the ice box, the resultant thick soup was filtered. Now concentrated hydrochloric acid (107.5 cc.) was slowly added until maximal precipitation of the fibrin was obtained. The supernatant liquid, neutral to litmus, was decanted and the precipitate washed a second time with water. After the supernatant fluid was again decanted, the residue was dehydrated by the addition of 3 volumes of 95 per cent alcohol. This was filtered off, and the white precipitate was washed with more alcohol and preserved under it. Thus 196 Gm. of precipitate was obtained, which was found by analysis to be 41.5 per cent solid.

The total product was treated with dry potassium hydroxide (3.46 Gm.) and the whole diluted by the addition of 500 cc. of water to a manageable syrup. The mixture was neutral to phenolphthalein until more water was added; then it turned alkaline.

Such readily reproducible proteinates were the "standard" from which the experiments now to be described proceeded. Though essentially pure potassium fibrinate, they still carried some chlorine (also chemically bound to the proteins for reasons elucidated elsewhere²).

A quantitative study of the decomposition of hydrogen peroxide by such materials under identical circumstances was accomplished via a battery of gas-washing bottles of uniform design, connected by a delivery tube to a pneumatic trough holding calibrated receptacles for the reception of gas. By such a scheme did Burge⁸ study quantitatively the catalase content of tissue. An interesting parallel between his studies and ours lies in the fact that the "concentrations" of tissue, of hydrogen peroxide and of other factors employed by him are of the same order of magnitude as those used by us.

IV

Pure (potassium) fibrinate is without catalytic activity. Alone or diluted with water, it fails to decompose any of the 5 cc. of 30 per cent hydrogen peroxide added to it (tube 1 of table 1). "Activation" is accomplished by the addition of alkali, as tubes 2, 3 and 4 illustrate.

It is not the alkali added which decomposes the hydrogen peroxide. This is demonstrated in table 2. Fibrinate, therefore, is "catalase," as an equivalent of alkali added to water is not.

8. Burge, W. E.: Am. J. Physiol. **41**:153, 1916.

For maximal yield of oxygen an optimal concentration of alkali is necessary. As ordinarily put, correct p_H is called for. This is shown in table 1. The fact is reillustrated in table 3. Here sodium hydroxide has replaced potassium hydroxide, and a higher concentration of sodium hydroxide is needed to give a maximal amount of gas. Though the

TABLE 1.—*Experiment Demonstrating That Pure (Potassium) Fibrinate Is Without Catalytic Activity Until Alkali Is Added to It*

Tube	Mixture*	Cubic Centimeters of Gas Yielded in Given Number of Minutes			
		15 Min.	30 Min.	45 Min.	60 Min.
1	5 cc. fibrinate + 40 cc. H ₂ O (control).....	0	0	0	0
2	5 cc. fibrinate + 39.5 cc. H ₂ O + 0.5 cc. 2N KOH.....	85	130	150	165
3	5 cc. fibrinate + 39 cc. H ₂ O + 1 cc. 2N KOH.....	100	150	180	200
4	5 cc. fibrinate + 38 cc. H ₂ O + 2 cc. 2N KOH.....	80	135	170	190

* The fact that 5 cc. of 30 per cent hydrogen peroxide is present in all these mixtures is omitted from this and subsequent tables for the sake of brevity.

TABLE 2.—*Experiment Demonstrating That It Is Not the Added Alkali Which Decomposes the Hydrogen Peroxide*

Tube	Mixture	Cubic Centimeters of Gas Yielded in Given Number of Minutes			
		15 Min.	30 Min.	45 Min.	60 Min.
1	5 cc. fibrinate + 39 cc. H ₂ O + 1 cc. 2N KOH.....	90	140	165	180
2	44 cc. H ₂ O + 1 cc. 2N KOH.....	0	2	5	8

TABLE 3.—*Experiment of Table 1 Repeated with Use of Sodium Hydroxide Instead of Potassium Hydroxide to Show Relation of Concentration and Kind of Alkali to Yield of Gas (Oxygen)*

Tube	Mixture	Cubic Centimeters of Gas Yielded in Given Number of Minutes			
		15 Min.	30 Min.	45 Min.	60 Min.
1	5 cc. fibrinate + 40 cc. H ₂ O (control).....	0	0	2	2
2	5 cc. fibrinate + 39 cc. H ₂ O + 1 cc. 2N NaOH.....	90	135	170	190
3	5 cc. fibrinate + 38 cc. H ₂ O + 2 cc. 2N NaOH.....	110	160	195	230
4	5 cc. fibrinate + 37 cc. H ₂ O + 3 cc. 2N NaOH.....	100	150	195	230

two alkalis are equally "strong," the sodium, in the lower concentrations at least, is not so effective an "activator" of the "ferment" as potassium.

The yield of oxygen is proportionate to the concentration of fibrinate. Table 4 illustrates the relation of the "concentration" of a "ferment" to the quantity of its activity.

The alkalinized fibrinate catalyzes an "infinite" amount of substrate. Tables 5 and 6 show how the same fibrinate mixture (with

TABLE 4.—*Effect of Decreasing the Concentration of the "Ferment" on the Yield of Gas*

Tube	Mixture	Cubic Centimeters of Gas Yielded in Given Number of Minutes		
		30 Min.	45 Min.	60 Min.
1	10 cc. fibrinate + 34 cc. H ₂ O + 1 cc. 2N KOH.....	330	380	420
2	5 cc. fibrinate + 39 cc. H ₂ O + 1 cc. 2N KOH.....	200	230	260
3	1 cc. fibrinate + 43 cc. H ₂ O + 1 cc. 2N KOH.....	50	60	80

TABLE 5.—*Catalysis of an "Infinite" Amount of the Substrate by the Fibrinate, Sodium Hydroxide Being Used as the Alkalizer*

Tube	Mixture	Cubic Centimeters of Gas Yielded in Given Number of Minutes or Hours						
		15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.	24 Hr.
1	5 cc. fibrinate + 40 cc. H ₂ O (control)	0	0	5	18	25	...	100
2	5 cc. fibrinate + 38 cc. H ₂ O + 2 cc. 2N NaOH.....	105	155	190	220	245	...	540
3	5 cc. fibrinate + 36 cc. H ₂ O + 4 cc. 2N NaOH.....	120	190	240	280	310	...	515
4	5 cc. fibrinate + 34 cc. H ₂ O + 6 cc. 2N NaOH.....	120	205	260	300	325	...	480
5	5 cc. fibrinate + 32 cc. H ₂ O + 8 cc. 2N NaOH.....	130	200	248	280	305	...	465

After 24 hours, 5 cc. more of 30 per cent H₂O₂ was added to each of these mixtures. The yields of gas were:

0	0	0	0	0	0	...
45	65	95	120	145	165	...
60	100	140	170	205	230	...
65	115	165	195	220	250	...
120	175	220	255	280	300	...

When another 24 hours had elapsed, a third addition of 5 cc. of H₂O₂ was made. The evolution of gas was as follows:

0	0	0	0	0	0	...
15	45	65	80	92	105	...
50	80	110	145	170	195	...
65	100	135	170	190	220	...
110	180	230	265	300	320	...

TABLE 6.—*Catalysis of an "Infinite" Amount of the Substrate by the Fibrinate, Ammonium Hydroxide Being Used as the Alkalizer*

Tube	Mixture	Cubic Centimeters of Gas Yielded in Given Number of Minutes or Hours						
		15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.	24 Hr.
1	5 cc. fibrinate + 40 cc. H ₂ O (control)	0	5	10	20	25	30	...
2	5 cc. fibrinate + 38 cc. H ₂ O + 2 cc. 2N NH ₄ OH.....	65	100	115	130	145	160	...
3	5 cc. fibrinate + 36 cc. H ₂ O + 4 cc. 2N NH ₄ OH.....	70	90	115	135	150	165	...
4	5 cc. fibrinate + 34 cc. H ₂ O + 6 cc. 2N NH ₄ OH.....	80	120	140	170	180	195	...
5	5 cc. fibrinate + 32 cc. H ₂ O + 8 cc. 2N NH ₄ OH.....	90	140	160	190	205	225	...

At the end of 24 hours a second 5 cc. of 30 per cent H₂O₂ was added to the aforementioned mixtures:

0	0	0	0	0	0	18
2	7	13	20	25	30	390
4	10	25	35	45	50	280
8	25	40	55	68	80	500
15	40	60	75	90	105	520

And at the end of 48 hours, a third such addition was made:

0	0	0	0	0	0	...
0	4	10	20	25	30	...
2	18	25	40	50	60	...
15	30	40	55	70	80	...
10	30	50	65	80	95	...

sodium hydroxide used as the alkalizing agent in the first and ammonium hydroxide in the second) repeats on successive days the same decomposition of hydrogen peroxide. Only these points need to be observed in regarding the tables: In absolute rate of decomposition the order of the active positive "ions" is $K > Na > NH_4$; also, the reaction mixtures "weaken," less oxygen being liberated on the second and third days of the experiment than on the first in the unit time.

Salts inhibit the activity of alkalized fibrinate to varying degrees, dependent on their concentration and kind. The effects of increasing

TABLE 7.—*Inhibition of the Activity of an Alkalized Fibrinate by a Salt (Potassium Chloride)*

Tube	Mixture	Cubic Centimeters of Gas Yielded in Given Number of Minutes			
		15 Min.	30 Min.	45 Min.	60 Min.
1	5 cc. fibrinate + 39 cc. H ₂ O + 1 cc. 2N KOH (control)....	105	140	175	200
2	5 cc. fibrinate + 39 cc. H ₂ O + 1 cc. 2N KOH + 0.1491 KCl	99	135	165	185
3	5 cc. fibrinate + 39 cc. H ₂ O + 1 cc. 2N KOH + 0.2982 KCl	85	120	150	175
4	5 cc. fibrinate + 39 cc. H ₂ O + 1 cc. 2N KOH + 0.5964 KCl	72	110	140	160

TABLE 8.—*More "Poisonous" Effect of a Heavy Metal (Barium Chloride) on the Alkalized Fibrinate*

Tube	Mixture	Cubic Centimeters of Gas Yielded in Given Number of Minutes or Hours			
		30 Min.	45 Min.	60 Min.	16 Hr.
1	10 cc. fibrinate + 34 cc. H ₂ O + 1 cc. 2N KOH + 0.208 Gm. BaCl ₂ ·2H ₂ O.....	10	17	20	220
2	5 cc. fibrinate + 39 cc. H ₂ O + 1 cc. 2N KOH + 0.208 Gm. BaCl ₂ ·2H ₂ O.....	5	8	10	160
3	1 cc. fibrinate + 44 cc. H ₂ O + 1 cc. 2N KOH + 0.208 Gm. BaCl ₂ ·2H ₂ O.....	0	0	0	50

concentrations of a salt, chemically unreactive with any constituent of the ferment mixture, are illustrated in table 7. "Salting" obviously "preserves" the "catalase" mixture.

Heavier metals are more specifically "poisonous." The effects of barium are shown in table 8, the fibrinate mixtures being identical with those of table 4, but with barium chloride added in an amount not quite the equivalent of the 1 cc. of twice normal potassium hydroxide present in each. Any optical sign of precipitation of the barium as the hydroxide was absent.

Table 9 illustrates the effect on an active fibrinate-alkali mixture of the addition of chemical equivalents of a series of different chlorides.

The hardly perceptible suppressive effects of potassium become almost complete as calcium, mercury, magnesium and barium take its place.

These differences between the effects of different basic radicals hold also for the acid radicals. Tubes 2 and 3 of table 10 show how potassium chloride is less effective than potassium sulfate. In the remaining tubes the greatly suppressive activity of dipotassium sulfide is particu-

TABLE 9.—*Increasingly Suppressive Effects of Chemical Equivalents of a Series of Chlorides Added to the Fibrinate-Alkali Mixture*

Tube	Mixture	Cubic Centimeters of Gas Yielded in Given Number of Minutes or Hours			
		30 Min.	45 Min.	60 Min.	16 Hr.
1	5 cc. fibrinate + 39 cc. H ₂ O + 1 cc. 2N KOH (control)	80	100	120	400
2	5 cc. fibrinate + 39 cc. H ₂ O + 1 cc. 2N KOH + 0.1492 KCl	80	100	115	390
3	5 cc. fibrinate + 39 cc. H ₂ O + 1 cc. 2N KOH + 0.111 CaCl ₂	5	7	8	70
4	5 cc. fibrinate + 39 cc. H ₂ O + 1 cc. 2N KOH + 0.270 HgCl ₂	0	0	2	50
5	5 cc. fibrinate + 39 cc. H ₂ O + 1 cc. 2N KOH + 0.095 MgCl ₂	0	0	0	10
6	5 cc. fibrinate + 39 cc. H ₂ O + 1 cc. 2N KOH + 0.244 BaCl ₂ ·2H ₂ O	0	0	0	140 (?)

TABLE 10.—*Differing Effects of a Series of Acid Radicals Added to the Fibrinate-Alkali Mixture*

Tube	Mixture	Cubic Centimeters of Gas Yielded in Given Number of Minutes					
		15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.
1	5 cc. fibrinate + 39 cc. H ₂ O + 1 cc. 2N KOH (control)	85	125	150	170	185	200
2	5 cc. fibrinate + 39 cc. H ₂ O + 1 cc. 2N KOH + 1 cc. 2N KCl	90	125	155	175	190	205
3	5 cc. fibrinate + 39 cc. H ₂ O + 1 cc. 2N KOH + 1 cc. 2N K ₂ SO ₄	60	90	110	130	140	160
1	5 cc. K fibrinate + 39 cc. H ₂ O + 1 cc. 2N KOH (control)	65	95	120	140	160	170
2	5 cc. K fibrinate + 38 cc. H ₂ O + 1 cc. 2N KOH + 1 cc. 2N KNO ₃	50	90	110	130	145	160
3	5 cc. K fibrinate + 38 cc. H ₂ O + 1 cc. 2N KOH + 1 cc. 2N KClO ₃	60	90	112	130	145	155
4	5 cc. K fibrinate + 38 cc. H ₂ O + 1 cc. 2N KOH + 1 cc. 2N K ₂ S	20	20	30	35	40	50
5	5 cc. K fibrinate + 38 cc. H ₂ O + 1 cc. 2N KOH + 1 cc. 2N phenol	150	220	295	350	400	430

larly noteworthy. Phenol, on the other hand, nearly doubles the rate of gas evolution.

In order not to lengthen this paper unduly, we merely state that the addition of the various potassium salts of the acetic series of acids to such mixtures as those named showed the lowermost members (formate through caproate) to inhibit as does ordinary sodium chloride. But by the time the caprate is reached, the decomposition of hydrogen peroxide is actually furthered (owing in our opinion to the fact that at the caprylate level these salts at the concentrations of water here

employed pass from solutions in the water to the hydrated soaps themselves, then in a physicochemical state identical with that of the protein ferment).⁹

V

We think that the experiments just detailed show how a protein, the fibrin from blood, is, by proper treatment with alkali, converted into a system which in every way behaves like the tissue extracts prepared for the study of catalase. Itself inactive, fibrin accelerates the decomposition of hydrogen peroxide as soon as it is properly alkalinized. In the terminology of ferments, a proferment is thus changed to a ferment. The material is active in the same low concentration in which recognized catalase is active, and it exhibits an optimal p_{H} . Acids retard or obliterate the decomposition effects of the fibrinate according to their concentration and kind just as they do this to standard catalase extracts.¹⁰ The same fibrinate system produces an "infinite" amount of chemical change, corresponding in its behavior with any of the biochemist's "ferments." All salts reduce this activity, and where specific effects have been noted in the instance of specific salts in the study of catalase, parallelism is again complete. Even though large differences appear among the findings of different authors in this total field (due, no doubt, to the difference in the source of their catalase and in the history of their preparations), they agree in fundamentals. Thus ammonium always proves destructive at one end of a long series of salts carrying different basic radicals. The light metal salts show themselves least "poisonous"; they are followed in crescendo by the alkaline earths¹¹ and the heavier metals.¹² A similar harmony of effect on catalase and effect on our fibrinate exists for the acid radicals—the halogens being least inhibitory, with various "weak" organic acids occupying a middle zone, and the oxyacids proving in general most obviously "poisonous."¹³

We assume quite naturally that the described variations in the activity with which a protein decomposes hydrogen peroxide are associated with changes in its colloid chemical state as induced through

9. Fischer, M. H.: *Soaps and Proteins: Their Colloid Chemistry in Theory and Practice*, New York, John Wiley & Sons, Inc., 1921, p. 21.

10. Euler, H.: *Beitr. z. chem. Phys. u. Path.* **7**:1, 1905. Senter, G.: *Ztschr. f. physiol. Chem.* **44**:257, 1903.

11. Faitelowitz, A.: *Zur Kenntnis Der Milchkatalyse Des H_2O_2* , Dissert., Heidelberg, 1904 (not available in the original).

12. Euler, H.: *Ark. f. kemi* **2**:222, 1907. Favre, W.: *Biochem. Ztschr.* **33**:32, 1911.

13. For a summary of the voluminous and chiefly Scandinavian literature, see Oppenheimer, C.: *Die Fermente*, ed. 5, Leipzig, Georg Thieme, 1926, vol. 2, p. 1841.

changes in its surroundings (temperature, acidity or alkalinity, salt content, etc.). The protein must be made neither too soluble in water (as after treatment with ammonia) nor yet too little hydratable (as after treatment with a heavy metal). The greatest activity seems associated with the maximal degree of hydration *not* correlated with the maximal degree of dispersion (in "molecular solution" the protein again becomes inactive). This statement repeats in essence the view of the physical state of all ferments maintained by Fodor.¹⁴ But our findings contribute further to what has long been a subject of debate. Reasoning from observed biochemical behavior, a majority has concluded that most ferments must be protein. Waentig and Gierisch¹⁵ urged this for catalase specifically. To this we agree. As such, then, they become intimate blocks in the structure of living matter, in other words of that hydrated mass known as protoplasm.

SUMMARY

A potassium fibrinate is prepared from a shelf stock of blood fibrin and shown to behave like the catalase extracts of tissues or tissue juices. On the basis of qualitative and quantitative parallelisms a simple protein is thus stated to be the chemical equivalent of a ferment.

14. Fodor, A.: *Das Fermentproblem*, Dresden, Theodor Steinkopff, 1922, p. 172.

15. Waentig, P., and Gierisch, W.: *Fermentforsch.* **1**:165, 1916.

CASEIN AS CATALASE

MARTIN H. FISCHER, M.D., Sc.D.

AND

WERNER J. SUER, M.S.

CINCINNATI

In a previous paper¹ it was shown how fibrin by proper chemical treatment may be made to assume all the activities of a "ferment." Through proper "alkalinization" this inactive protein from blood decomposed hydrogen peroxide as so much "catalase." These paragraphs detail how another globulin, the casein of milk, functions in identical fashion. The laboratory setup was as in the earlier experiments.

For "stock," 12.5 Gm. of casein (Harris) was soaked in 15 cc. of water overnight, and then 10 cc. of normal sodium hydroxide was added. The product was a thick paste. When diluted with water to 150 cc., it contained in the unit volume a quantity of protein approximately half of that of the same unit volume of the "standard" syrup of fibrin used in the studies cited.¹

Sodium caseinate alone does not decompose hydrogen peroxide. Table 1 illustrates this fact as also the "activation" of the caseinate to "catalase" through the addition of alkali.

Table 2 exhibits an "optimal p_H " for the activity of the "ferment." It lies at a concentration of alkali less than that represented in mixture 5. The relation of the concentration of the "ferment" to the quantity of activity is illustrated in table 3.

Further proof that the decomposition of the hydrogen peroxide in these mixtures is more than the effect of their concentration of caseinate, p_H or "alkalinity" is furnished in table 4. Here equivalent concentrations of different alkalis were employed. Ammonium hydroxide is obviously less effective than potassium hydroxide or sodium hydroxide; while all three stand far above the hydroxides of calcium and barium, which, in fact, show themselves to be violently inhibitory.

How addition of increasing increments of a neutral salt (potassium chloride) to an active caseinate mixture of constant composition decreases its capacity to decompose hydrogen peroxide is apparent in table 5.

The effects of different acid radicals with constancy in the other components of the mixtures are shown in table 6.

From the Laboratory of Physiology, University of Cincinnati.

1. Fischer, M. H., and Suer, W. J.: Arch. Path., this issue, p. 815.

TABLE 1.—*Experiment Showing That Caseinate Acquires Catalytic Activity Only on Alkalinization*

Tube	Mixture*	Cubic Centimeters of Gas Evolved After Given Number of Minutes					
		15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.
1	10 cc. sodium caseinate + 35 cc. H ₂ O (control)	0	0	0	0	0	0
2	10 cc. sodium caseinate + 34 cc. H ₂ O + 1 cc. 2N NaOH.....	55	90	120	150	170	195
3	10 cc. sodium caseinate + 33 cc. H ₂ O + 2 cc. 2N NaOH.....	73	135	185	230	270	300
4	10 cc. sodium caseinate + 32 cc. H ₂ O + 3 cc. 2N NaOH.....	100	170	227	275	315	350
5	10 cc. sodium caseinate + 31 cc. H ₂ O + 4 cc. 2N NaOH.....	120	210	270	320	360	390
Controls showed that the decomposition of hydrogen peroxide was not a matter merely of the addition of alkali. Pure alkali in the concentration represented in mixture 2 yielded the following amounts of gas:							
		2	3	15	20	25	30

* The fact that each mixture includes 5 cc. of 30 per cent hydrogen peroxide is omitted from this and subsequent tables for the sake of brevity.

TABLE 2.—*Experiment Demonstrating the Relation of the Concentration of Alkali to the Catalytic Activity of the Caseinate-Alkali Mixture*

Tube	Mixture	Cubic Centimeters of Gas Evolved After Given Number of Minutes or Hours						
		15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.	24 Hr.
1	10 cc. sodium caseinate + 35 cc. H ₂ O (control)	0	0	0	0	0	0	0
2	10 cc. sodium caseinate + 33 cc. H ₂ O + 2 cc. 2N NaOH.....	25	90	120	150	180	200	510
3	10 cc. sodium caseinate + 31 cc. H ₂ O + 4 cc. 2N NaOH.....	42	150	210	255	290	325	510
4	10 cc. sodium caseinate + 29 cc. H ₂ O + 6 cc. 2N NaOH.....	100	180	240	275	310	335	520
5	10 cc. sodium caseinate + 27 cc. H ₂ O + 8 cc. 2N NaOH.....	125	190	235	265	295	300	500

TABLE 3.—*Experiment Demonstrating the Relation of the Concentration of Caseinate to the Catalytic Activity of the Caseinate-Alkali Mixture*

Tube	Mixture	Cubic Centimeters of Gas Evolved After Given Number of Minutes					
		15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.
1	20 cc. sodium caseinate + 24 cc. H ₂ O + 1 cc. 2N KOH.....	35	65	95	120	150	170
2	15 cc. sodium caseinate + 29 cc. H ₂ O + 1 cc. 2N KOH.....	30	60	85	105	130	150
3	10 cc. sodium caseinate + 34 cc. H ₂ O + 1 cc. 2N KOH.....	30	50	70	95	115	130
4	5 cc. sodium caseinate + 39 cc. H ₂ O + 1 cc. 2N KOH.....	30	50	70	85	100	112
5	2.5 cc. sodium caseinate + 41.5 cc. H ₂ O + 1 cc. 2N KOH.....	25	40	60	70	90	100

TABLE 4.—*Experiment Showing Differing Degrees of Catalytic Activity When Caseinate Is Alkalinized by Different Alkalis*

Tube	Mixture	Cubic Centimeters of Gas Evolved After Given Number of Minutes					
		15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.
1	10 cc. sodium caseinate + 35 cc. H ₂ O (control).....	0	0	0	0	0	0
2	10 cc. sodium caseinate + 35 cc. N/25 NH ₄ OH.....	6	25	40	55	65	75
3	10 cc. sodium caseinate + 35 cc. N/25 NaOH.....	12	35	52	65	80	100
4	10 cc. sodium caseinate + 35 cc. N/25 KOH.....	20	45	60	80	90	110
5	10 cc. sodium caseinate + 35 cc. N/25 Ca(OH) ₂	0	0	0	0	0	0
6	10 cc. sodium caseinate + 35 cc. N/25 Ba(OH) ₂	0	0	0	0	0	0

TABLE 5.—*Experiment Showing Increasingly Inhibitory Effect on Catalytic Activity of Alkalinized Caseinate with Additions of Increasing Amounts of a Neutral Salt*

Tube	Mixture	Cubic Centimeters of Gas Evolved After Given Number of Minutes						
		15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.	105 Min.
1	10 cc. sodium caseinate + 34 cc. H ₂ O + 1 cc. 2N KOH (control).....	10	35	55	70	90	100	115
2	10 cc. sodium caseinate + 34 cc. H ₂ O + 1 cc. 2N KOH + 0.1442 KCl.....	10	25	45	60	80	90	100
3	10 cc. sodium caseinate + 34 cc. H ₂ O + 1 cc. 2N KOH + 0.2884 KCl.....	10	25	40	55	70	82	95
4	10 cc. sodium caseinate + 34 cc. H ₂ O + 1 cc. 2N KOH + 0.4326 KCl.....	4	20	35	50	60	75	85
5	10 cc. sodium caseinate + 34 cc. H ₂ O + 1 cc. 2N KOH + 0.5768 KCl.....	2	10	30	40	50	60	70

TABLE 6.—*Differing Effects on Catalytic Activity of Alkalinized Caseinate with Addition of Different Acid Radicals*

Tube	Mixture	Cubic Centimeters of Gas Evolved After Given Number of Minutes					
		15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.
1	10 cc. sodium caseinate + 34 cc. H ₂ O + 1 cc. 2N KOH (control).....	22	45	70	87	105	120
2	10 cc. sodium caseinate + 34 cc. H ₂ O + 1 cc. 2N KOH + 0.0194 KSCN.....	32	60	80	100	115	130
3	10 cc. sodium caseinate + 34 cc. H ₂ O + 1 cc. 2N KOH + 0.0244 KClO ₃	22	40	60	80	100	112
4	10 cc. sodium caseinate + 34 cc. H ₂ O + 1 cc. 2N KOH + 0.0202 KNO ₃	22	40	60	78	100	112
5	10 cc. sodium caseinate + 34 cc. H ₂ O + 1 cc. 2N KOH + 0.013 KCN.....	10	25	42	57	70	85
1	10 cc. sodium caseinate + 34 cc. H ₂ O + 1 cc. 2N KOH (control).....	35	75	95	120	135	155
2	10 cc. sodium caseinate + 34 cc. H ₂ O + 1 cc. 2N KOH + 0.0332 KI.....	105	190	255	305	345	375
3	10 cc. sodium caseinate + 34 cc. H ₂ O + 1 cc. 2N KOH + 0.012 KH ₂ AsO ₄	40	70	100	120	120	160
4	10 cc. sodium caseinate + 34 cc. H ₂ O + 1 cc. 2N KOH + 0.0196 KC ₂ H ₃ O ₂	35	70	90	110	135	155
5	10 cc. sodium caseinate + 34 cc. H ₂ O + 1 cc. 2N KOH + 0.0162 KCNO.....	35	60	80	90	118	130

In order not to lengthen our protocols unduly we shall merely state that in oft-repeated series of experiments we found the inhibiting activity of different acid radicals or of different basic radicals to follow the general order discovered in the instance of fibrin functioning as catalase. For example, five mixtures containing different amounts of sodium caseinate (20 cc. to none) with constant amounts of potassium hydroxide and barium chloride yielded no gas whatsoever. The same proved true of calcium chloride. An exception was encountered in the instance of mercury: Its bichloride *increased* the decomposing effects of casein on hydrogen peroxide.

SUMMARY

Ordinary casein, itself inactive in the decomposition of hydrogen peroxide, may be made to function as catalase through the addition of alkali. But only the hydroxides of the lighter metals prove active in this regard. The conditions which make for the inhibition, or suppression, of an otherwise active mixture are identical with those which reduce catalase activity in biochemical extracts.

EXPERIMENTALLY INDUCED BENIGNANCY OF NEOPLASM

II. THE EFFECT OF TREATMENT WITH AN ESTROGEN AND OF CASTRATION OF THE HOST

IRA T. NATHANSON, M.D.

AND

WILLIAM T. SALTER, M.D.

BOSTON

In a previous number of this journal Gardner, Smith, Strong and Allen¹ reported the production of sarcoma with an estrogen (theelin), administered subcutaneously. This interesting demonstration of carcinogenic effect suggests that an excess of estrogenic substance in the animal body favors malignant growth. It is possible, however, to demonstrate another effect of estrogenic substance: inhibition of the growth of sarcoma.

In previous papers from this laboratory dealing with susceptibility to inoculated sarcoma² the importance of the animal host was emphasized. It is clear that "malignancy" is not a property of the tumor alone. "Malignancy" is, rather, the reflection of the host's lack of immunity (or resistance) against neoplastic growth.³ This resistance can be altered in experimental animals to varying degrees. Thus, in parallel experiments, inoculated pedigreed mice may show (a) no growth of tumors, (b) growth of "benign" tumors or (c) growth of "malignant" tumors.^{2b} What factors influence this varying immunity?

One factor which may influence the degree of immunity is sex. Bittner⁴ reported a difference in reaction to a transplanted tumor in males and females. Likewise, Andervont⁵ in inoculation experiments

From the Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University.

Aided by grants to William T. Salter from the International Cancer Research Foundation and a grant from the Committee on Research in Endocrinology of the National Research Council.

1. Gardner, W. U.; Smith, G. M., Strong, L. C., and Allen, E.: *Arch. Path.* **21**:504, 1936.

2. (a) Salter, W. T., and Oster, R.: *J. Clin. Investigation* **15**:466, 1936.

(b) Oster, R. H., and Salter, W. T.: *Am. J. Cancer* **32**:422, 1938.

3. Throughout this paper the term "immunity" is used in a specialized sense to indicate resistance against tumor growth on the part of the host.

4. Bittner, J. J.: *Am. J. Cancer* **16**:322, 1932.

5. Andervont, H. B.: *Pub. Health Rep.* **47**:1859, 1932.

with sarcoma 180 found that more than twice as many females became immune as males.⁶ This finding suggests that androgenic and estrogenic hormones may influence the development of resistance against neoplasm. It is the purpose of this report to describe the effect of the administration of an estrogenic substance in excess and of castration on immunity to mouse sarcoma 180.

EXPERIMENTAL METHOD

Pedigreed mice (Bagg albino, strain A) were inoculated in the tail by the procedure of Andervont,⁵ and the tumors were amputated after two to four weeks. The animals were then reinoculated in the groin. The growing tumors were measured with calipers in three diameters. These measurements were made twice weekly according to the procedures of Bischoff and Maxwell⁷ and Schrek.⁸ Control animals from the same generation of albino mice were inoculated in the groin without previous "immunization." These controls received tissue from the same tumor as the test animals. Each test animal was inoculated alternately with a control animal. The control tumors were measured simultaneously with the corresponding test tumors.

As a control strain, black mice of Bar Harbor strain C-57 were used. This strain becomes "immune" much less readily than the albino strain A.

The estrogen was administered according to the following schemes:

Scheme 1.—Test animals received daily subcutaneous injections of theelin⁹ (estrone [3 hydroxy 17-keto-1,3,5-estratriene]) in peanut oil. The oil was injected into the back, far from the proposed site for inoculation of the tumor. After two weeks each animal was inoculated in the tail with tumor. Daily injections of the estrogen were continued. After two to four weeks the tail tumors were amputated. Each animal was then inoculated in the groin with the test tumor. Injections of the estrogen were continued without interruption. As soon as the groin tumors became palpable, measurements were begun.

"Immunity control" animals were treated in like manner except that peanut oil (0.1 cc.) was used in place of the solution of theelin.

"Virulence control" animals were completely untreated animals of the same generation. Each of these was inoculated in the groin with the test tumor. Thus the integrity of the tumor was assured.

Such controls were used in all subsequent schemes as needed.

Scheme 2.—Test animals received daily subcutaneous injections of the estrogen in peanut oil. After two weeks each was inoculated in the tail with tumor. Daily injections of the estrogen were continued. After two to three weeks the tail tumors were amputated. The injections of theelin were then discontinued. Each animal was inoculated in the groin with the test tumor. Measurements were made as outlined in scheme 1.

"Immunity control" animals and "virulence control" animals were maintained as in scheme 1.

Scheme 3.—Test animals received daily injections of theelin in peanut oil. After two weeks the injections were discontinued and each mouse was inoculated in the groin with the test tumor. No inoculation in the tail was made in this group.

6. This sex difference is not found in all strains of mice.

7. Bischoff, F., and Maxwell, L. C.: *Am. J. Cancer* **27**:87, 1936.

8. Schrek, R.: *Am. J. Cancer* **28**:345, 1936; *Am. J. Path.* **12**:525, 1936.

9. The theelin was contributed by Parke, Davis and Company.

Appropriate "immunity controls" and "virulence controls" were maintained.

Scheme 4.—Each animal was inoculated in the groin with the test tumor. Thereupon test animals received daily subcutaneous injections of theelin in peanut oil. Injections were continued throughout the period during which measurements were made.

Appropriate "virulence controls" were maintained simultaneously.

Scheme 5.—Test animals and "immunity controls" were inoculated in the tail with tumor. After two or three weeks the tail tumors were amputated. Immediately scheme 4 was started.

Scheme 6.—Castration was performed one month prior to inoculation of the groin with the test tumor. Afterward measurements of the inoculated tumors were made twice weekly.

Simultaneous "virulence controls" were maintained.

DOSAGE OF THE ESTROGEN

The daily dose of theelin varied from 50 to 200 international units. Other experiments performed in this laboratory on castrated animals indicate that 1 mouse unit of theelin (or estrone) is equivalent to approximately 1 international unit of crystalline estrone (or theelin). This is the amount required to produce estrus in the castrated animal. Accordingly, the doses used in these experiments must be regarded as more than ample.

EXPERIMENTAL DATA

The experimental results represent studies of 632 animals. The data were originally formulated as tumor growth curves, corresponding to about a month's time. Each tumor was ordinarily measured in three diameters twice a week. In order to condense the 9,000 values thus obtained, only mean diameters are presented in this report. Furthermore, in most instances only the mean diameters at maximal size (i. e., after three to four weeks' growth) are presented. The data are arranged in relation to certain specific questions.

1. *Does an excess of estrogen per se influence the growth of sarcoma 180?*

This problem was attacked according to scheme 4. The data are summarized in table 1.

Experiment 1.—Two groups of black adult males of the C-57 strain received different dosages of theelin. Group 1 received 50 international units per day; group 2, 200 units per day. Corresponding testicular weights are recorded as evidence of the effect of the estrogen. Appropriate "virulence controls" are shown.

Experiment 2.—A single group of black adult males received 200 units of theelin per day.

Experiment 3.—A single group of black adult females, C-57 strain, received 200 units of theelin per day.

Experiment 4.—A single group of albino adult males, strain A, received 100 units of theelin daily.

Experiment 5.—A single group of albino adult males received 200 units of theelin daily.

Experiment 6.—A single group of albino adult females, strain A, received 200 units of theelin daily.

From table 1 it will be seen that in experiments 1, 2 and 3 there was slight but definite inhibition of tumor growth. In fact, experiments

TABLE 1.—*Does Theelin per Se Influence Tumor Growth? (Scheme 4)*

	Controls for Virulence	Dose of Estrogen	
		50 I. U. Group 1	200 I. U. Group 2
Experiment 1: black males, C-57			
Number of animals.....	12	12	14
Mean tumor diameter at 3½ weeks, mm.....	16.9	11.0	9.0
Mean testicular weight, mg.....	84.4	72.7	56.9
Experiment 2: black males, C-57			
Number of animals.....	16	..	18
Mean tumor diameter at 3½ weeks, mm.....	17.2	12.5
Mean tumor diameter at 4 weeks, mm.....	20.3	15.0
Experiment 3: black females, C-57			
Number of animals.....	18	..	18
Mean tumor diameter at 3 weeks, mm.....	15.8	13.3
Mean tumor diameter at 4 weeks, mm.....	20.7	15.5
Summary of experiments 1, 2 and 3			
Number of animals.....	46	12	50
Mean tumor diameter at 3 weeks, mm.....	16.5	11.0	11.8
Experiment 4: albino males, strain A		100 I. U.	
Number of animals.....	14	14	
Mean tumor diameter at 3 weeks, mm.....	17.0	14.6	
Mean tumor diameter at 4 weeks, mm.....	20.3	20.5	
Experiment 5: albino males, strain A			
Number of animals.....	23	25	
Mean tumor diameter at 3 weeks, mm.....	13.0	11.0	
Mean tumor diameter at 4 weeks, mm.....	16.6	15.7	
Experiment 6: albino females, strain A			
Number of animals.....	24	18	
Mean tumor diameter at 3 weeks, mm.....	11.1	12.4	
Mean tumor diameter at 4 weeks, mm.....	15.3	17.5	
Summary of experiments 4, 5 and 6			
Number of animals.....	61	57	
Mean tumor diameter at 3 weeks, mm.....	13.4	12.4	
Mean tumor diameter at 4 weeks, mm.....	17.0	17.5	

TABLE 2.—*Effect of Experiment with Theelin on Body Weight*

	Virulence Controls	Dose of Estrogen	
		50 I. U.	200 I. U.
Male mice, C-57.....	10	10	10
Mean weight at start, Gm.....	25.7	29.9	30.0
Mean weight after 3 weeks, Gm.....	30.7	29.8	38.3

1 and 2 showed little or no overlapping between the controls and the treated groups, as indicated in figure 1. Furthermore, the inhibition paralleled testicular weight. It should be noted that our use of mean diameter as an index of growth tends to minimize differences in tumor growth. The differences shown in figure 1 would be more marked if volumes had been calculated from the three diameters, $V = 4/3 \pi abc$, or

by the prolate-spheroid formula ($4/3 \pi ab^2$), as used by Emge and Murphy.¹⁰

The animals were healthy mice of the black C-57 strain. Indeed, table 2 presents the gross weights of the estrogen-treated animals, which were not less than the control values. These weights indicate that cachexia was not a factor in the experiment.

At first sight one might be tempted to generalize and assert that the estrogen inhibited tumor growth directly. In the albino animals, however, practically no inhibiting effect was observed after the injection of the estrogen, as shown in table 1. In fact, the females of experiment 6

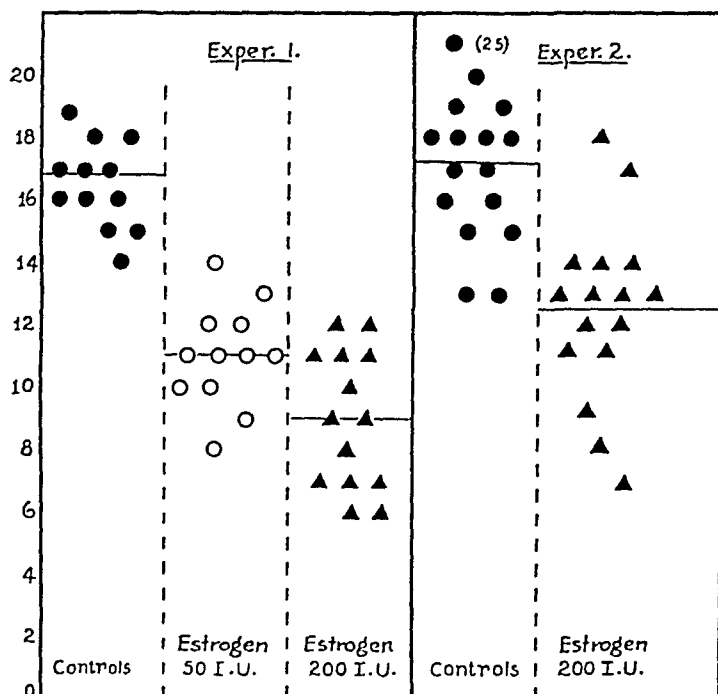


Fig. 1.—Effect of huge doses of theelin on size of tumors in mice. The values at the left represent the mean diameter of tumors in millimeters.

bore larger tumors than the controls. As will appear in table 3, further data substantiate this negative result.

In general, the answer to question 1 is apparently: No. The estrogen per se does not markedly inhibit the growth of sarcoma 180.

There remains to be explained the definite effect produced in the black animals of experiments 1, 2 and (possibly) 3. This result probably can be explained satisfactorily on the basis of an enhancement of the immune response, as described later under question 3. In short, the effect appears to be an indirect one.

10. Emge, L. A., and Murphy, K. M.: *Am. J. Obst. & Gynec.* **32**:593, 1936.

2. Does the estrogen (in excess) enhance the efficiency of "immunization"?

The problem was attacked according to schemes 1, 2 and 3. It can be considered in several phases:

(a) Preliminary treatment with the estrogen might make the animal more resistant to tumor growth (scheme 3).

(b) The estrogen might enhance the efficiency of the tail inoculation in producing "immunity" (scheme 2).

(c) A continuous excess of the estrogen (scheme 1) might be more effective than either (a) or (b) alone.

(d) In "immunized" animals the estrogen might enhance the previously induced inhibition of tumor growth (scheme 5).

As shown in table 3, possibility *a* is excluded. Experiment 1 on black males and experiment 2 on black females both gave negative results. It will be recalled, likewise, that table 1 shows no effect in albino animals, to which the estrogen was administered while test tumors were growing.

As to possibility *b*, little effect was demonstrated. Experiment 3 in black males and experiment 4 in black females showed no striking enhancement of immunity resulting from tail inoculation combined with simultaneous administration of theelin. Both of the groups subjected to tail inoculation had fewer "takes" and showed smaller tumors than the "virulence controls."

As to possibility *c*, the experimental data are definitely positive.

Experiments 5a and 5b in black males, C-57 strain, showed definitely fewer successful "takes" than their "immunity controls" or than the animals in experiments 3 and 4. It could also be shown that a definite effect on tumor size was obtained with high doses of theelin. In general, the average size of test tumors in animals receiving high doses of the estrogen was smaller than that of test tumors in animals receiving low doses. Of course, the test tumors of both groups were much smaller than those in the "virulence controls," i. e., the completely untreated. Furthermore, the relative diminution in size, compared with that in the "virulence controls," was more striking than that found in animals without tail inoculation (see *d*, below). The simultaneous shrinking of testicular weight is of interest as collateral evidence of estrogen effect.

In albino males, strain A, experiment 6 showed a striking complete resistance to the inoculated tumor. In short, no test tumors developed. It must be conceded that possibly the tumors used in experiments 6a and 6b were not particularly virulent.

Theelin, then, did definitely enhance the final outcome of immunization.

TABLE 3.—Does Theelin Enhance Immunity?

	Estrogen Treatment		Estrogen Treatment		Estrogen Treatment		Estrogen Treatment	
	Controls	Estro- gen Alone	Tail Treat- ment Plus Estrogen	Controls	Estro- gen Alone	Tail Treat- ment Plus Estrogen	Controls	Estro- gen Alone
	Viru- lence	Immunity		Viru- lence	Immunity		Viru- lence	Immunity
Experiment 1, Scheme 3 Black Males, C-57								
Number of animals.....	12	12	12	12	..	24	24
Mean tumor diameter at 3 wk., mm...	15.1	16.0	14.8	15.2	..	15.0	15.6
Mean tumor diameter at 4 wk., mm...	20.3	20.7	20.3	19.7	..	20.3	20.3
Experiment 2, Scheme 3 Black Females, C-57								
Number of animals.....	12	12	12	12	..	24	24
Mean tumor diameter at 3 wk., mm...	15.1	16.0	14.8	15.2	..	15.0	15.6
Mean tumor diameter at 4 wk., mm...	20.3	20.7	20.3	19.7	..	20.3	20.3
Experiment 3, Scheme 2 Black Males, C-57								
Number of animals.....	10	15	15	10	15	15	20	30
Mean tumor diameter at 3 wk., mm...	15.1	10.2	10.7	14.3	11.4	11.0	15.0	10.8
Mean tumor diameter at 4 wk., mm...	20.3	13.9	13.4	20.3	15.7	15.1	20.3	14.8
Number of takes.....	9*	10	9	9*	9	8	18*	19
Percentage of takes.....	90*	66	60	90*	60	53	90*	63
Experiment 4, Scheme 2 Black Females, C-57								
Number of animals.....	10	15	15	10	15	15	20	30
Mean tumor diameter at 3 wk., mm...	15.1	10.2	10.7	14.3	11.4	11.0	15.0	10.8
Mean tumor diameter at 4 wk., mm...	20.3	13.9	13.4	20.3	15.7	15.1	20.3	14.8
Number of takes.....	9*	10	9	9*	9	8	18*	19
Percentage of takes.....	90*	66	60	90*	60	53	90*	63
Experiment 5a, Scheme 1 Black Males, C-57								
Number of animals.....	6	15	12	6	9	12	12	24
Mean tumor diameter at 3½ wk., mm.	19.3	11.5	12.5	17.0	10.5	10.5	18.2	11.1
Mean testicular weight, mg.....	90	..	57	87	..	61	87	..
Number of takes.....	6	6	5	6	6	4	12	12
Percentage of takes.....	100	40	42	100	60	33	100	50
Experiment 5b, Scheme 1 Black Males, C-57								
Number of animals.....	6	15	12	6	9	12	12	24
Mean tumor diameter at 3½ wk., mm.	19.3	11.5	12.5	17.0	10.5	10.5	18.2	11.1
Mean testicular weight, mg.....	90	..	57	87	..	61	87	..
Number of takes.....	6	6	5	6	6	4	12	12
Percentage of takes.....	100	40	42	100	60	33	100	50
Experiment 5a and 5b Combined								
Number of animals.....	6	15	12	6	9	12	12	24
Mean tumor diameter at 3½ wk., mm.	19.3	11.5	12.5	17.0	10.5	10.5	18.2	11.1
Mean testicular weight, mg.....	90	..	57	87	..	61	87	..
Number of takes.....	6	6	5	6	6	4	12	12
Percentage of takes.....	100	40	42	100	60	33	100	50

Experiment 6a, Scheme 1 Albino Males, Strain A			Experiment 6b, Scheme 1 Albino Males, Strain A			Experiments 6a and 6b Combined		
Number of animals.....	12	12	10	16	16
Mean tumor diameter at 3½ wk., mm.	10.3	0	17.6	8.9	0
Number of takes.....	10	0	10	6	0
Percentage of takes.....	100	0	100	37	0
Second Inoculation†								
Number of animals.....	10	0	10	10†	10†	52	16	..
Mean tumor diameter at 3½ wk., mm.	20.0	0	9*	9.6	0	19	9.1	..
Number of takes.....	10	0	90*	2	0	61*	8	28
Percentage of takes.....	100	0	90*	20	0	98*	50	0
Third Inoculation†								
Number of animals.....	10	0	10	8†	10†
Mean tumor diameter at 3½ wk., mm.	20.3	0	19.8	0	0
Number of takes.....	9*	0	10	0	0
Percentage of takes.....	90*	0	100	0	0
Grand total of takes, percentage.....	90*	0	97*	50	0
Experiment 7, Scheme 5 Black Males, C-57								
Number of animals.....	10	15	11	12	12
Mean tumor diameter at 4 wk., mm.	20.3	12.5	15	13.6	10.1
Number of takes.....	9*	8	11	6	2
Percentage of takes.....	90*	53	100	67	17
Experiment 8, Scheme 5 Black Females, C-57								
Number of animals.....	10	9	12	12	12
Mean tumor diameter at 4 wk., mm.	20.3	9	12	13.6	10.1
Number of takes.....	9*	6	6	12	2
Percentage of takes.....	90*	100	67	100	17
Experiment 9a, Scheme 5 Albino Males, Strain A								
Number of animals.....	10	10	10	10	13
Mean tumor diameter at 4 wk., mm.	19.6	18.7	6.1	18.2	7.3
Number of takes.....	10	10	5	10	5
Percentage of takes.....	100	100	33	100	38
Experiment 9b, Scheme 5 Albino Males, Strain A								
Number of animals.....	10	12	10	13	13
Mean tumor diameter at 4 wk., mm.	20.0	17.4	10.2	18.2	7.3
Number of takes.....	10	11*	0	10	5
Percentage of takes.....	100	93*	75	100	38
Experiment 9c, Scheme 5 Albino Males, Strain A								
Number of animals.....	10	10	10	10	10
Mean tumor diameter at 4 wk., mm.	20.0	22.1	16.5	21.1	18
Number of takes.....	10	10	8	10	10.1
Percentage of takes.....	100	100	50	100	28

* A second inoculation showed that susceptibility was really 100 per cent. In other words, the failure to take was due to a lapse in technique.
† Immune animals re-inoculated. A new group of virulence controls was used.

* A second inoculation showed that susceptibility was really 100 per cent. In other words, the failure to take was due to a lapse in technique.
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As to possibility *d*, one would logically predict a positive effect; i. e., since possibility *c* minus possibilities *a* and *b* equals possibility *d*, the last should be the important feature. That this was indeed the case can be seen from table 3. Experiments 7 and 8 on black adult animals, strain C-57, indicated a definite increase in the number of completely immune animals. There was likewise a difference in size between those test tumors which did develop and the tumors in the "immunity controls." Of course, both were smaller than the tumors in the "virulence controls." The results agree rather well with those of experiment 5 as contrasted

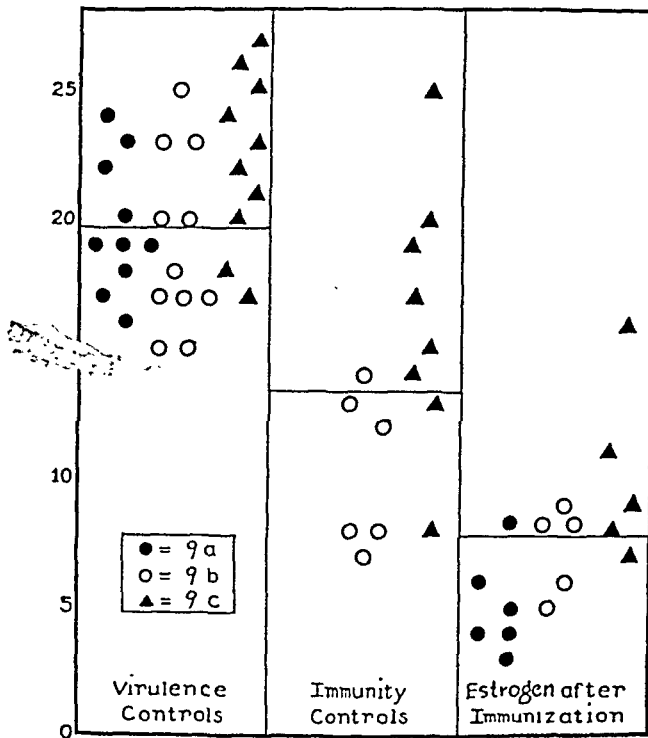


Fig. 2.—Effect of huge doses of theelin on size of tumors in tumor-immunized mice. The values at the left represent the mean diameter of tumors in millimeters.

with those of experiments 3 and 4. Experiments 9 a, b, c on albino adult males, strain A, gave similar results, as shown in figure 2.

In summary, the complete answer to question 2 is as follows: The estrogen does not increase markedly the efficiency of the immunizing procedure. After completion of the immunization, however, the estrogen markedly accentuates its effect.

Table 4 gives the results of statistical analysis of several experiments, appropriately combined. Only the immunized animals, with and without estrogen, have been compared. The probability that the difference reported is fortuitous is about 3 in 100 in individual groups. This figure neglects two other features which make for even less uncertainty, i. e.,

the considerable percentage of completely immune animals and the greater security of repeated observations.

It is known, in general, that some groin tumors provoke "immunity" against themselves as they grow. Thus Andervont¹¹ reported the frequent regression of sarcoma 37 after this tumor had attained a considerable size in strain M mice. Likewise sarcoma 180 grows and regresses in strain I mice.⁴ No such regression of sarcoma 180 occurs ordinarily in the strains used in the present experiments. In partially immunized animals of albino strain A however, such regression does occur. Furthermore, as shown in table 1, experiments 1 to 3, the response of the black C-57 animals to theelin suggests that the estrogen enhanced a slight immune response which otherwise might have escaped notice.

TABLE 4.—*Statistical Analysis Indicating Possibility that Results After Injections of Theelin Were Mere Chance*

Experimental Combinations	Immune Controls		Animals Treated with Theelin After Immunization		n	s	t	P
	Number	Mean Diameter of Tumors, Mm.	Number	Mean Diameter of Tumors, Mm.				
I, 1 and 2.....	28	17.0	30	10.8	56	2.30	10.26	0.000
III, 5a and 5b.....	12	11.0	5	7.5	15	2.42	2.72	0.01(7)
III, 7 and 8.....	14	12.1	6	9.3	18	2.35	2.44	0.03
III, 9b and 9c.....	15	13.6	10	8.7	23	4.43	2.71	0.01(3)

n = degrees of freedom; t = difference of means divided by standard error of this difference, adjusted for n; P = probability of a difference as great or greater than that occurring by chance alone. (Fisher, R. A.: *Statistical Methods for Research Workers*, ed. 6, London, Oliver & Boyd, 1936, chap. 5. Mellor, J. W.: *Higher Mathematics for Students of Physics and Chemistry*, ed. 4, New York, Longmans, Green & Co., 1931, chap. 9.)

3. *What is the effect of castration on the "immune" response?*

This question is pertinent because gonadectomy in females reduces the natural supply of estrogenic substance.¹² One might expect, therefore, that the castrated female host would be less resistant to tumor growth.

The problem of castration was attacked according to scheme 6. Further study will be necessary in view of conflicting reports by Emge, Murphy and Schilling¹³ and by Bischoff and Maxwell.⁷ It is interesting, however, that our preliminary results are not inconsistent with the findings given earlier in this communication. In table 5 experiment 1 on black animals, strain C-57, indicates that tumors borne by the castrated female hosts were slightly larger than those in the controls. In experi-

11. Andervont, H. B.: *Pub. Health Rep.* **52**:1885, 1937.

12. Frank, R. T.; Goldberger, M. A., and Salmon, U. J.: *Proc. Soc. Exper. Biol. & Med.* **33**:615, 1936. Nathanson, I. T.: Unpublished data.

13. Emge, L. A.; Murphy, K. M., and Schilling, W.: *Proc. Soc. Exper. Biol. & Med.* **38**:21, 1938.

ment 2 there is perhaps an equivocal tendency in the same direction. The effect is not very striking, but the amounts of theelin involved are very much less than in the earlier experiments. In general, among the males there seems to be no striking difference from the controls, although a few test tumors were unusually large.

According to Murphy and Sturm,¹⁴ the effect of castration varies with time. It may be that hypophysial hormones are involved in this phenomenon, as suggested by Druckrey.¹⁵ Our few results are reported merely to show that the observed experimental effects of castration and of treatment with theelin, respectively, are not inconsistent.

LONGEVITY OF ANIMALS WITH ARTIFICIALLY BENIGN TUMORS

Sarcoma 180 is a highly malignant growth which kills the animal host soon after inoculation. For example, in a census of 131 inoculated control animals, all but a few were found to be dead eight weeks after

TABLE 5.—Does Castration Influence Immunity?

	Experiment	Controls C-57		Oastrated Mice C-57	
		Males	Females	Males	Females
Number of animals.....	1	6	6	12	12
Mean tumor diameter at 3 weeks, mm.....		18.0	17.0	19.5	22.0
Mean tumor diameter at 4 weeks, mm.....		21.0	19.0	25.0	27.0
Average deviation at 4 weeks, mm.....		±2.7	±1.0	±3.3	±1.2
Number of animals.....	2	15	15	15	15
Mean tumor diameter at 3 weeks, mm.....		20.1	14.0	18.5	18.0
Mean tumor diameter at 4 weeks, mm.....		22.0	19.8	21.8	23.5
Average deviation at 4 weeks, mm.....		±1.7	±3.8	±2.2	±2.8

inoculation. In fact, 24 had died four weeks after inoculation and 89 by the sixth week. Thus 68 per cent were dead in a few weeks. A census of 29 partially immune animals in which small tumors grew showed a striking contrast; 2 had died at ten weeks, 1 at three months and 4 at five months. Thus after five months 76 per cent of the animals were living. In some, indeed, the tumors had regressed. This longevity of the host is striking evidence that artificially induced benignancy is real.

It should be noted that the huge doses of theelin used in our earlier experiments presumably influenced the pituitary, as reported by Cramer and Horning.¹⁶ In interpreting the action of the estrogen, therefore, one must bear in mind the possibility of profound indirect effects. Provided that large doses of theelin inhibit pituitary function, a secondary inhibition of tumor growth might result. Such an effect was reported by Ball and Samuels¹⁷ after hypophysectomy.

14. Murphy, J. B., and Sturm, E.: J. Exper. Med. **42**:155, 1925.

15. Druckrey, H.: Arch. f. exper. Path. u. Pharmakol. **181**:174, 1936.

16. Cramer, W., and Horning, E. S.: Lancet **1**:1056, 1936.

17. Ball, H. A., and Samuels, L. T.: Am. J. Cancer **32**:50, 1938.

COMMENT

These results are consistent with the concept² that "malignancy" is a graded condition depending on a balance between tumor and host. The reciprocal relationship between the neoplasm and the host's normal tissue can be altered by artificial variations in the immunization technic.⁶ It can be altered likewise by endocrinologic changes in the host, as demonstrated in this report.

The literature contains many references to estrogen as an aggravator of malignant disease.¹⁸ With few exceptions,¹⁹ most of these citations have to do with neoplastic degeneration of primary or secondary sex organs. The observations reported herewith, therefore, are of special interest for two reasons: First, they picture estrogen as a motivator for benignancy. Second, they have to do with the suppression of a tumor of nondescript fibrous tissue, unrelated to sex functions.

The mechanism, however, whereby such suppression is produced remains obscure. It need not be a direct sterol effect.²⁰ It may well involve a rearrangement of the balance between various endocrine glands of the host. Indeed, Cramer and Horning²¹ observed adrenal degeneration in pure strain mice subject to mammary cancer.

The literature also records other experiments designed to try the effect of androgenic and estrogenic substances on the growth of tumors. For example, Sugiura and Benedict²² and Wiesner and Haddow²³ reported such studies. Some of these were surveyed by Bischoff and Maxwell.⁷ The results of most of them were negative, in accord with our experiments on the effect of theelin per se. A few investigators, like Zondek, Zondek and Hartoch²⁴ and Nitta,²⁵ obtained positive results.

18. Lacassagne, A.: *Compt. rend. Acad. d. sc.* **195**:630, 1932. Gardner, W. U.; Smith, G. M.; Allen, E., and Strong, L. C.: *Arch. Path.* **21**:265, 1936. Gardner, W. U.; Smith, G. M.; Strong, L. C., and Allen, E.: *J. A. M. A.* **107**:656, 1936.

19. Notably the work of Gardner, Smith, Strong and Allen.¹

20. This statement does not mean that lipoids are unimportant in the production of cancer (cf. Claude, A.: *Science* **86**:294, 1937). Nor does it imply that immunity is not directly associated with the malignant process. It is interesting that, according to Haddow and his collaborators, carcinogenic agents produced inhibition of the growth of transplantable tumors, as well as of general body growth (Haddow, A., and Robinson, A. M.: *Proc. Roy. Soc., London, s.B* **122**:442, 1937. Haddow, A.; Scott, C. M., and Scott, J. D.: *ibid.* **122**:477, 1937).

21. Cramer, W., and Horning, E. S.: *Nature, London* **139**:196, 1937; *J. Path. & Bact.* **44**:633, 1937.

22. Sugiura, K., and Benedict, S. R.: *Am. J. Cancer* **18**:583, 1933.

23. Wiesner, B. P., and Haddow, A.: *Nature, London* **132**:97, 1933.

24. Zondek, H.; Zondek, B., and Hartoch, W.: *Klin. Wchnschr.* **11**:1785, 1932.

25. Nitta, Y.: *Jap. J. Obst. & Gynec.* **19**:90, 1936; abstracted, *Am. J. Cancer* **31**:112, 1937.

The experiments presented herewith go far toward reconciling the apparent discrepancies between several investigators, some of whom relied on physiologic changes or doses of physiologic magnitude, while others (e. g., Zondek, Zondek and Hartoch) used large amounts of estrogen.

Among the negative effects reported for theelin are those of Emge, Murphy, and Schilling,¹³ who studied the effect of this estrogen on transplantable mammary rat adenofibroma. Their findings in white rats were corroborated by our results in albino mice of strain A *when preliminary immunization was omitted*. Thus the apparent discrepancies in the literature can be explained on two grounds: that of dosage and that of immune reaction.

It should be emphasized that this work is not concerned with the *origin* of neoplasms. It has to do merely with their continued existence and growth. The animal host is regarded as an animated culture medium which may be made, to a varying degree, favorable or unfavorable to tumor growth. This concept was clearly established early in this century.²⁶

SUMMARY

Pedigreed mice were artificially "immunized" against sarcoma 180 by preliminary inoculation in the tail. In such animals the "immunity" was enhanced by large doses of theelin, given while the test tumors were developing. The number of completely immune animals was increased. Furthermore, in nonimmune animals, strain C-57, the rate of growth of the implanted tumors was somewhat slowed by the administration of theelin while these test tumors were growing. In albino animals, strain A, however, the latter effect was not noted. The effect of the estrogen on the growth of the tumors, therefore, was probably not a direct inhibition on the tumors. The estrogen secondarily enhanced a primary inhibiting mechanism.

Castration of females possibly decreased resistance to tumor growth, but the effect was slight at best.

These results in toto suggest that the fate of an inoculated tumor is partly determined by the endocrinologic status of the host.

26. Clowes, G. H. A.: Bull. Johns Hopkins Hosp. **16**:130, 1905. Bashford, E. F.; Murray, J. A., and Cramer, W.: Proc. Roy. Soc., London, s.B **79**:164. 1907. Lumsden, T.: Am. J. Cancer **15**:563, 1931; Lancet **2**:814, 1929.

EPITHELIAL FUNCTIONAL REJUVENATION OBSERVED IN THE MUCOUS CELLS OF THE GASTRO- INTESTINAL TRACT AND THE PARIETAL CELLS OF THE STOMACH

NICHOLAS W. POPOFF, M.D.

ROCHESTER, N. Y.

Throughout the epithelial layer of the alimentary tract from the esophagus to the anus are found peculiar cells, scattered here and there among the other elements, which are known in histology as enterochromaffin or argentochrome cells. Their histogenesis, though a subject of research for more than fifty years, is still a matter of debate, and their physiologic role remains unknown. It is generally held that these cells are of entodermal origin. There are, however, investigators who claim ectodermal origin for them. Neither initial nor terminal stages of their histogenic cycle have been traced with certainty. There is no generally accepted concept concerning their function. The range of theories runs from restrained presumptions to fantastic assumptions. Some believe that they are externally secreting (exocrine) digestive glands. Others consider them as absorbing cells. On account of their topographic distribution and their specific reducing power they are regarded by some as glands of internal secretion. Their endocrine function is linked with the metabolism of carbone hydrate, the production of secretin, the secretion of epinephrine, the formation of antianemic factors and other processes. In the opinion of some investigators, argentochrome cells discharge their secretion neither into the lumen of the alimentary tract nor into the blood and lymph streams, and their secretion acts only locally on the nerves. The latter hypothetic action is designated as a neurocrine function. There is no better critical review of this controversial subject than that by Macklin and Macklin,¹ and further discussion of other theories may be omitted here. In closing their review on the nebulous condition of present day knowledge of the argentochrome cells Macklin and Macklin come to the conclusion that the most that can be said of the function of these cells is that it con-

From the Department of Pathology, Highland Hospital.

This work was aided by a grant from the Committee on Scientific Research of the American Medical Association.

1. Macklin, C. C., and Macklin, M. T.: *The Intestinal Epithelium*, in Cowdry, E. V.: *Special Cytology: The Form and Functions of the Cell in Health and Disease*, ed. 2, New York, Paul B. Hoeber, Inc., 1932, vol. 1, p. 233.

stitutes an engaging problem, solution of which is a challenge to investigators.

From analysis of the vast literature on this subject it is apparent that progress in this field of research is hindered chiefly by limitations on the methods employed in the study of this problem. So far only histologic methods are available for the study of the physiologic and pathologic aspects of these cells, and the main and recommended methods of silver impregnation are complicated, time consuming and of doubtful specificity. Cumbersome and uncertain, they remain practically inapplicable to the study of argentaffin cells under various experimental conditions. Morphologic studies made with these methods cover the subject in a most exhaustive way, and nothing new can be expected from reexamination of this problem by these means.

In discussing the reasons for the limitations of the methods in use the following eight points of criticism are justifiable:

1. Trinitrophenol-formaldehyde solution (Bouin's picroformol fluid) is recommended as the fixative of choice, but repeated warnings are given to the effect that fixatives containing mercury bichloride or potassium bichromate are detrimental and should not be employed. For topographic and general cytologic studies Bouin's fluid is indeed valuable but is not generally recognized to be of value for fine histologic studies. It destroys much of the cell content and thus gives an incorrect picture of the cells. An ingredient of this fixative that is most injurious to the finer constituents of the cell is acetic acid. It is common knowledge among modern workers that a cell fixed in a solution containing acetic acid has a more "raked out" appearance than one fixed in a solution from which acetic acid has been omitted. This applies not only to cell granules but to the appearance of the ground cytoplasm, nucleoli and chromatin filaments (Lee²). By hemolyzing the erythrocytes acetic acid destroys their reducing power. Bouin's fluid is of no value for fine hematologic studies, and certainly it is not the fixative of choice for studies of intracellular structures.

2. Ammoniacal silver is considered to be the only proper solution for use in demonstrating silver-reducing granules. The ways of preparing this solution are not governed by exact standards of chemical procedure, and the time required for impregnation is long and beyond objective control. With the methods most commonly employed now, the step of silver impregnation alone requires from thirty-six to forty-eight hours.

3. The formation of metallic sols prepared with reduction methods, their dispersity, their stability and their precipitation depend on the type

2. Lee, A. B.: *The Microtometist's Vade-Mecum: A Handbook of the Methods of Animal and Plant Microscopic Anatomy*, ed. 10, Philadelphia, P. Blakiston's Son & Co., 1937.

of reducer employed. It might be expected, then, that by trying a new and more effective reducer different results could be obtained from histochemical impregnation.

4. In all present day methods, counterstaining is done after silvering, and it is questionable whether this way of counterstaining can be considered dependable in tracing various morphologic changes concomitant with the life cycle of the epithelial silver-reducing cell.

5. It is reasonable to expect that the initial formation, accumulation and disappearance of silver-reducing granules must be preceded or followed by detectable changes in the nuclei of corresponding cells. The methods in use offer no selective technic for the study of this aspect of the problem.

6. Simultaneous and practically unavoidable impregnation of various forms of wandering cells, macrophages, lymphocytes in transition, granular leukocytes and their forerunners is one of the most serious defects of methods employed now. This technical defect has served as a main source of confusion and has led a number of investigators to incorrect conclusions concerning the histogenesis and function of epithelial silver-reducing cells. It is obvious that the technical problem of tinctorial differentiation between epithelial silver-reducing cells and the various silver-reducing cells of mesodermal and ectodermal origin is of utmost importance.

7. It is claimed that the granules of Paneth cells are not impregnable with the silver methods commonly used. A reexamination of this question with the aid of a new technic is of considerable importance.

8. If specific diphenols are responsible for silver and chrome reduction, trials with the reduction of other metals are more than justifiable.

ADVANTAGEOUS FEATURES OF THE NEW TECHNIC

As a result of the investigations to be reported here, the objectionable points just discussed may be corrected by the use of a new technic based on entirely new principles. Introductory statements concerning the important features of the new methods, arranged in an order corresponding to that of the discussed eight points, are as follows:

1. In these newly devised methods the trinitrophenol-formaldehyde solution is replaced with a fixative introduced by Helly.

2. Ammoniacal silver is replaced with silver nitrate solution and the time of silver impregnation is reduced from forty hours to ten minutes.

3. A new and specially adjusted combination of hydrazine hydrate and water blue has been prepared and serves as the reducer of choice. Applied for five minutes, this solution acts simultaneously as a reducer and as a counterstain.

4. Counterstaining with safranin and with eosin is done before silvering, and the results offer new information pertaining to the life cycle of the silver-reducing cells.

5. With the combined eosin-potassium bichromate-water blue-silver technic, the silver-reducing cells show in their various phases of activity certain important nuclear changes which are not demonstrable with ordinary methods.

6. The rapid and easily controlled technic of silver impregnation and the use of certain chemicals, applied before silvering, have served to differentiate various tissue elements by disclosing either comparative degrees of silver-reducing power or loss of this power.

7. Paneth cells in a certain phase of their secretory activity show with the new technic granules which give a silver reduction reaction.

8. It has been found that with a modified technic the other metals (mercury, gold, tellurium and bismuth) may give reduction reactions similar to those obtained with silver. Their tinctorial effect is not as good as that obtained with silver, but their histochemical significance is identical.

PRINCIPLES UNDERLYING THE NEW METHODS

Before describing the new methods it is desirable to discuss (1) the principles underlying them, (2) the types of reagents employed and (3) the tinctorial and impregnation results obtained with each method. With all these newly devised methods the fixative employed is Helly's fluid. The essential prerequisite is that the tissues must be perfectly fresh. The tissues are run through alcohols and embedded in accordance with the chloroform-paraffin method.

The principle of the first and main silver method is based on the reduction of silver nitrate with a hydrazine hydrate-water blue mixture. When the hydrazine hydrate ($\text{H}_2\text{N}-\text{NH}_2$) is added to the solution of water blue, there is formed immediately a yellowish leukocompound. Acting as a reducer, this leukocompound, on reoxidation and change of p_{H} , gives also an excellent effect of counterstaining. Hydrazine hydrate alone, applied in equivalent dilution, fails to give reductions similar to those obtained with the leukocompound. A leukocompound formed on heating the solution of water blue with zinc dust and applied to sections after silvering gives only faint traces of reduction. It is apparent, then, that the selective efficiency of the new reducer depends on a fitting combination in one solution of hydrazine hydrate and water blue. With this reducer, a solution of silver nitrate as weak as 0.04 per cent applied for ten minutes only is sufficient to impregnate epithelial silver-reducing granules. An important step, without which the methods described fail to work, is a thorough treatment of the sections with aqueous solution of iodine, and this must be done before silvering.

The main silver method impregnates in a specific way the granules of certain epithelial and mucous cells, the nuclei of certain mucous cells, the granules of certain parietal cells of the stomach, certain granules of Paneth cells, macrophages, certain leukocytes and the fine reticulum. Helly's fixative preserves excellently all the blood elements, and the method just described impregnates also the erythrocytes with an effect that is equal to that obtained with any good peroxidase reaction.

In the first variant of the main method sections are stained first with safranin. Then they are treated with silver nitrate solution and reduced with the hydrazine hydrate-water blue leukocompound. The unusual value of this method is that it stains the mucus of the goblet cells selectively and with tinctorial differentiations corresponding to the various phases of their life cycle. In line with the tinctorial differentiation of mucus it depicts also corresponding stages of cellular transformations which are associated with the appearance of the silver-reducing granules in the mucous cells.

In the second variant of the main method sections are stained first with the potassium bichromate-eosin-ammonium hydroxide mixture. Then they are treated with silver nitrate solution and reduced with the hydrazine hydrate-water blue leukocompound. Though both the dyes employed (eosin and water blue) are acid, the tinctorial effect obtained is such that eosin acts as a nuclear dye and stains only nuclei of certain mucous cells. The nuclei of other mucous cells either are impregnable with silver or appear violet blue, blue and light greenish blue. Comparing the tinctorial effect of this method with that of the main silver method, one perceives that the nuclear eosinophilia and the nuclear silver-reducing power shown by certain types of mucous cells are similar in their biologic significance. While the nuclei are silver reducing with the main method, with the second variant of that method the nuclei of the same types of mucous cells appear eosinophilic or silver reducing. In other words, these two methods are reciprocal. With this second variant the granules of eosinophilic leukocytes stain a deep red, and this facilitates cellular differentiation, which is rather difficult with the main silver method, as the latter impregnates the granules of these cells and the granules of true epithelial cells with almost equal intensity. The nuclei of active histiocytes, especially of those engaged in the formation of reticulum, are stained red with this variant method, while their fine protoplasmic processes appear black. This method offers also additional facilities for differential studies of the transformation forms derived from free and fixed elements of the mesenchyme.

The results obtained indicate that the common affinity of certain tissue elements for silver can be dissociated by a combination of various tinctorial and chemical factors. Of chemical factors, the p_H of the water used for washing sections before the silver bath and the strength of the

silver nitrate are of essential importance. Washing the sections before silvering in water of above p_H 7 intensifies or increases the impregnation phenomenon, while washing in water of below p_H 7 weakens it. If the wash water used before silvering is of p_H 7 and the time of silver impregnation is the same, the comparative degree of the silver-reducing power of each tissue element can be followed up by modifying the strength of the silver nitrate solution used. Thus, on decreasing the strength of the silver nitrate solution from 4 per cent to 0.04 per cent, the combination of the three methods described facilitates to a great extent the matter of cellular differentiation. In addition to this, the following simple test has been found of service: If sections are treated first with 30 per cent hydrogen dioxide in an alkaline medium for fifteen minutes and then the main silver method is applied, some epithelial cells lose and some lessen their argentaffinity, while the argentaffinity of mesenchymal elements remains intact.

Mercurous nitrate ($HgNO_2 \cdot H_2O$) in a 2 per cent aqueous solution applied for three to five minutes and reduced with the hydrazine hydrate-water blue leukocompound gives a reduction reaction which is equivalent to that obtained with silver. Similar reduction reactions are obtained on treating sections with this mercurous nitrate solution and reducing it with a 4 per cent aqueous solution of stannous chloride ($SnCl_2 \cdot 2H_2O$) or with ammonium sulfide (light hydrosulfide solution).

Sections treated with gold chloride solution and reduced with hydrazine hydrate leukocompound fail to show a selective gold reduction reaction. If, however, sections are treated first with 1 per cent potassium tellurite or with a 2 per cent bismuth nitrate-d-mannitol solution or with 50 per cent formic acid and are then reduced with the hydrazine hydrate-water blue leukocompound and toned afterward with gold chloride, reduction reactions take place in the cells which corresponds to those of epithelial argentaffin cells.

MATERIAL EXAMINED AND SCOPE OF EXPERIMENTATION

Simple in technic and selective in results, the methods described offer a new opportunity for studies of the morphologic and functional aspects of the silver-reducing cells of the gastrointestinal tract. In the present studies only rabbits and fresh human surgical material were used. The human surgical material examined included a large variety of inflammatory and neoplastic processes. The specificity and selective property of these newly devised methods depend essentially on the freshness of the tissues, and for this reason no human postmortem material was used in these studies. In this work entire attention was concentrated on subjects pertaining to the histophysiologic aspects of the epithelial silver-reducing cells, and material obtained from normal rabbits, and rabbits used for experiments formed the foundation of the investigation.

The purpose of these experiments was to investigate the response of the intestinal epithelial silver-reducing cells after local and general application of various chemical substances. This response was studied in conjunction with concomitant changes in other constituents of the mucosa. The same material was also used for studies of the terminal vascular system of the gastrointestinal tract,³ and for this reason extremely detailed studies of the entire wall were made in every instance. The stomach, small intestine, appendix and sigmoid (including the rectum) were studied in rabbits. Control tissues were taken from normal fasting animals kept on full vitamin diet, from animals at different stages of digestion and from animals kept on different types of diet.

TECHNIC OF EXPERIMENTS ON RABBITS TO ASCERTAIN EFFECT
OF APPLICATION OF CHEMICAL SUBSTANCES TO
GASTROINTESTINAL MUCOSA

The local effects of various chemical substances were studied in the following way:

The animal was kept fasting for twenty-four hours and was then anesthetized with ether. The abdomen was opened, and on both sides portions (loops) of the intestine were ligated with the least handling possible and away from blood vessels. The chemical substance was warmed to body temperature and introduced into the lumen with the aid of a syringe needle (G-24) and through a spot free from visible capillaries. Into an adjacent loop, made in a similar manner, as a control, was injected physiologic solution of sodium chloride. The needle was withdrawn without signs of hemorrhage or return leaking. In some comparative experiments two or three isolated loops were made in the same segment of the intestine, and into each loop a different substance was introduced. The abdomen was then closed with sutures and the animal, left alone without anesthetic, was kept alive for a period varying from ten minutes to one hour. On completion of the experiment the rabbit was disposed of either by an injection of air into the ear vein or by a blow over the neck. The abdomen was then reopened, the mesenteric blood vessels supplying the experimental and the control portions of the intestine were ligated, and the entire segment of intestine was removed gently and without loss of blood. In each instance the segment removed contained an adjacent part of the intestine into which the chemical substance had not been injected as a control.

The whole tissue was then placed in Helly's fixative for fifteen minutes. Each individual loop, including the control loop, was cut across into small segments; the lumen of each segment was washed out with Helly's fixative, and the trimmed segments were placed in fresh Helly's fixative for twenty-four hours. Control tissues also were fixed in this way. After being removed from the abdominal cavity, the intestinal loops were immediately cut across into small segments, and the content of each segment was washed out with 10 per cent formaldehyde and placed in Helly's fluid for twenty-four hours. Certain parts of the intestine, even after being removed from the abdominal cavity, remained sensitive to handling and to thermal shock. This necessitated controls with fixation performed *in situ*.

3. Popoff, N. W.: Arch. Path., to be published.

The latter was done by disposing of the animals in the manner already described and filling the abdominal cavity with the Helly fixative, which had been warmed to body temperature and introduced without touching the experimental and control portions of the intestine.

Experiments also were made with the object of studying the effects on the metallic reduction reaction itself of a delay in fixation of from ten minutes to four hours. These experiments were helpful in offering an opportunity for further study of the phenomenon of Mingazzini. The entire work reported here was based on examination of tissues with the aid of serial sections.

The following fifteen chemical substances were investigated with regard to their respective local effects on silver-reducing and other cells of the intestinal tract:

1. Epinephrine in solution (1:3,000 to 1:1,000)
2. Atropine (0.02 mg. dissolved in 5 cc. of distilled water)
3. Benzyl benzoate in solution (benzyl benzoate, 20 per cent; alcohol, 75 per cent), with 75 per cent and 40 per cent alcohol as parallel controls
4. Physostigmine (1 mg. dissolved in 5 cc. of distilled water)
5. Dextrose in solution (1:10)
6. Histamine in solution (1:10,000 to 1:1,000)
7. Lactic acid in solution (1:20)
8. Aqueous solution of iodine (in strength originally employed by Gram)
9. Magnesium sulfate in solution (1:10 and 1:5)
10. Sodium carbonate in solution (1:20)
11. Volatile oil of mustard U. S. P. (3 per cent in olive oil)
12. Ox bile (10 per cent dried *Bacto* ox bile in distilled water)
13. Pilocarpine (0.015 mg. dissolved in 5 cc. of distilled water)
14. Sodium nitrite (0.003 mg. dissolved in 5 cc. of distilled water)
15. Silver nitrate in solution (1:20)

In the study of general or indirect effects, the following substances, administered subcutaneously, were used:

1. Epinephrine, 0.5 mg.
2. Histamine, from 0.05 to 1 mg.
3. Pilocarpine, 0.5 and 1 mg.

Animals were disposed of at different intervals after injection, and tissues were taken care of in accordance with the technic already described.

HISTOLOGIC TECHNIC

Tissues were fixed in Helly's fluid for twenty-four hours, washed in running water for twenty-four hours, dehydrated in ascending alcohols (the time not exceeding thirty-six hours altogether), placed in chloroform for twelve hours and in chloroform saturated with paraffin twelve hours, passed through three changes (five hours each) of pure paraffin without beeswax and then embedded in the usual way.

Serial sections were attached to the slides with the aid of Masson's gelatin-dilute formaldehyde method (Masson⁴). To remove the paraffin, sections were run through three changes of xylene. On being taken out of the last change of xylene, the slides were plunged into 0.2 per cent pyroxylin in alcohol-ether for one to two minutes; they were removed and the excess of pyroxylin solution was drained off quickly, after which they were placed in 80 per cent alcohol. Devised by Regaud, this method of pyroxylinization assured perfect attachment of serial sections, which is essential in work with metallic impregnation. From the 80 per cent alcohol the sections were transferred to distilled water (p_H 7) for fifteen minutes (three changes of five minutes each), placed in Weigert's aqueous solution of iodine for twenty-five minutes, run through distilled water (p_H 7) for three minutes, 95 per cent alcohol for five minutes and distilled water (p_H 7) for three minutes, placed in 4 per cent sodium thiosulfate for ten minutes and washed in three changes of distilled water (p_H 7) for fifteen minutes altogether. The sections were then ready for staining and silver reduction.

The first or main method requires the following six reagents: (1) distilled water of p_H 7, (2) distilled water of p_H 6, (3) 4 per cent silver nitrate solution, (4) reducer, (5) 1 per cent acetic acid, freshly prepared each time to avoid contamination with molds, and (6) 4 per cent sodium thiosulfate.

The silver nitrate solution is made of Merck's blue label silver nitrate dissolved in freshly distilled water. The distilled water used is obtained with the aid of an all metal distiller, and no interference with metallic impregnation reactions is noticed. The stock solution of silver nitrate is stored in a perfectly clean bottle with a glass stopper and kept in a dark place. The working solution is made each time by filtering the required amount into a perfectly clean brown glass dropping bottle.

The reducer is prepared by adding to 10 cc. of aqueous 2 per cent water blue (*Wasserblau*-6B Extra P-Holborn) 10 drops of hydrazine hydrate (Eastman Kodak-P902 42 per cent in water). As the hydrazine hydrate is added drop by drop and the mixture shaken, the blue color of the water blue solution is rapidly changed to a light port wine color. This leukocompound is filtered into a perfectly clean brown glass dropping bottle, and if kept in a dark place and tightly closed with a well grounded glass stopper it does not deteriorate for quite a long time. In work reported in this paper a freshly prepared reducer was used each time.

The technic being simple and rapid, all procedures of the combined staining and reduction are carried out on the slide at room temperature, the only precaution being to avoid exposure to direct light.

Method 1.—This is the silver-water blue method. The steps are as follows:

1. Use a dropping bottle flood section prepared as directed in the foregoing text with 4 per cent silver nitrate and leave it on the slide for ten minutes.
2. Drain off the excess of silver solution and blot the slide with a pad of filter paper. When blotting the next slide, use a *clean space* on the filter pad.
3. Without letting the section become dry, flood it quickly with hydrazine hydrate-water blue leukocompound and leave this applied for five minutes. The section turns rusty brown and is covered with a multitude of minute bubbles.

4. Masson, P.: *Diagnostics de laboratoire: Tumeurs—diagnostics histologiques*, Paris, A. Maloine & Fils, 1923.

4. Wash the section by gently pouring distilled water of p_H 7 from a beaker, and after washing blow gently over the surface of the slide. Repeat this procedure quickly three times. The section turns yellowish green.

5. Place the slide in 1 per cent acetic acid, moving it up and down a few times, and leave it in the acid for five minutes. Here the section turns blue.

6. Place the slide in distilled water of p_H 7 and wash it in three changes of distilled water of p_H 7 for three minutes altogether.

7. Flood the section with 4 per cent sodium thiosulfate for four minutes.

8. Rinse and then place the slide in distilled water of p_H 6 for three minutes, moving it up and down a few times. During this time the excess of blue is removed. Then transfer the slide into a fresh change of distilled water of p_H 6 for seven minutes.

9. Dehydrate the section in 95 per cent alcohol for thirty seconds and follow with two changes of absolute alcohol of one minute each.

10. Prepare with xylene and balsam in the usual way.

Six slides can be handled easily at one time, the entire procedure taking forty-five minutes. Up to procedure 6 it is important each time to wipe off the end of the slide which is handled with metal forceps or to have the ends of the metal forceps well coated with paraffin. This procedure is to be used in all methods described.

Method 2.—This is the safranin-silver-water blue method.

1. After deparaffinization and treatment with aqueous solution of iodine, wash the sections as in the first method, with three changes of distilled water of p_H 7 for fifteen minutes.

2. Flood the sections with alcoholic safranin solution for ten minutes. This stain is made by diluting saturated alcohol-soluble safranin (Hollborn) with an equal part of distilled water of p_H 7. A 25 per cent aqueous solution of heat-saturated water-soluble safranin (Hollborn) may be used. Distilled water of p_H 7 is used in making safranin solutions.

3. After the treatment with safranin, wash the sections in three changes of distilled water of p_H 7 for three to five minutes altogether and then follow the technic of the first method from step 1 with only two modifications: (a) Instead of 4 per cent silver nitrate use 2 per cent silver nitrate, and (b) after slides are placed in 1 per cent acetic acid, keep them there for three minutes (here they give off the excess of safranin). Then transfer them into a fresh change of 1 per cent acetic acid and leave them in this for an additional three minutes.

Method 3.—This is the potassium bichromate-eosin-silver-water blue method.

1. Prepare sections for staining as described in the foregoing text.

2. Stain sections with the potassium bichromate-eosin-ammonium hydroxide mixture for twenty minutes. To make this mixture add to 7 cc. of 6 per cent aqueous potassium bichromate 3 cc. of 1 per cent yellowish water-soluble eosin (Hollborn), and to this mixture add 1 drop of ammonium hydroxide.

3. Using distilled water of p_H 7, rinse the sections and wash them with two changes of water for two minutes altogether.

4. Proceed now from step 1 of the first method. If the potassium bichromate-eosin mixture employed in this method is made without adding ammonium hydroxide, is filtered and applied, it gives somewhat different tinctorial and impregnation results, which are very instructive for comparative study.

The reagents used and the basic principles of the seven variants of the reduction tests made with mercury, gold, tellurium and bismuth have been discussed previously in this paper. Whether impregnation in these tests is combined with staining or is employed alone, the pretreatment with aqueous solution of iodine is essential.

RESULTS FROM STUDY OF THE SIGMOID

In describing the results of these investigations each anatomic part of the alimentary tract is considered separately. In this report the sigmoid is chosen first for the following reasons: (1) Compared with other parts of the alimentary tract, it has the simplest histologic structure and (2) its secretory product comes from one cellular source and does not contain significant amounts of enzymes. The sigmoid is devoid of villi; its glandular coat is lined with simple columnar epithelium, rich in goblet cells, and, as a rule, no cells of Paneth are found. Lymphoid follicles are scattered singly, and this permits studies on selected parts of the intestines devoid of this tissue-complicating structure.

The sigmoid of a full grown young rabbit that has been kept fasting for twenty-four hours is taken as the standard normal control. The results obtained with the newly developed methods are constant and uniform. The commonly observed type of silver-reducing cell in the sigmoid is that which is described in textbooks of histology as an argentaffin cell. With its broad base adhering closely to the basement membrane, this cell has the form of a conical flask with a cytoplasmic continuation directed toward the lumen. In certain instances only this type of silver-reducing cell is found (fig. 1).⁵ It is the easiest type of cell to demonstrate, as it gives positive impregnation even with a very weak solution of silver nitrate (0.04 per cent applied for ten minutes). Figure 1 shows that these cells are equally numerous in the tip and at the bottom of a fold.

Neither the type nor the number of argentaffin cells is the same everywhere. Some sections show a considerable number of these cells scattered here and there, and in others none are found. On examining a vast variety of serial sections one notes that coincident with impregnation of granules in argentaffin cells there is silver impregnation of granules in certain mucous cells (fig. 2 *A*). On applying a gradually decreasing concentration of silver nitrate (4 to 0.04 per cent) one finds that the impregnation shown by the granules of these mucous cells cannot be attributed to a fault in technic. In some mucous cells the granules are small and few (fig. 2 *A*, right side of the field). In other cells they are large, numerous and segregated chiefly in the perinuclear zone (fig. 2 *A*, central and left glands). The nuclei in these cells are pushed to the base, their structure appears indistinct, and they also show the

5. The photomicrographs were prepared by Mr. M. C. Orser, of the school of medicine of the University of Rochester.

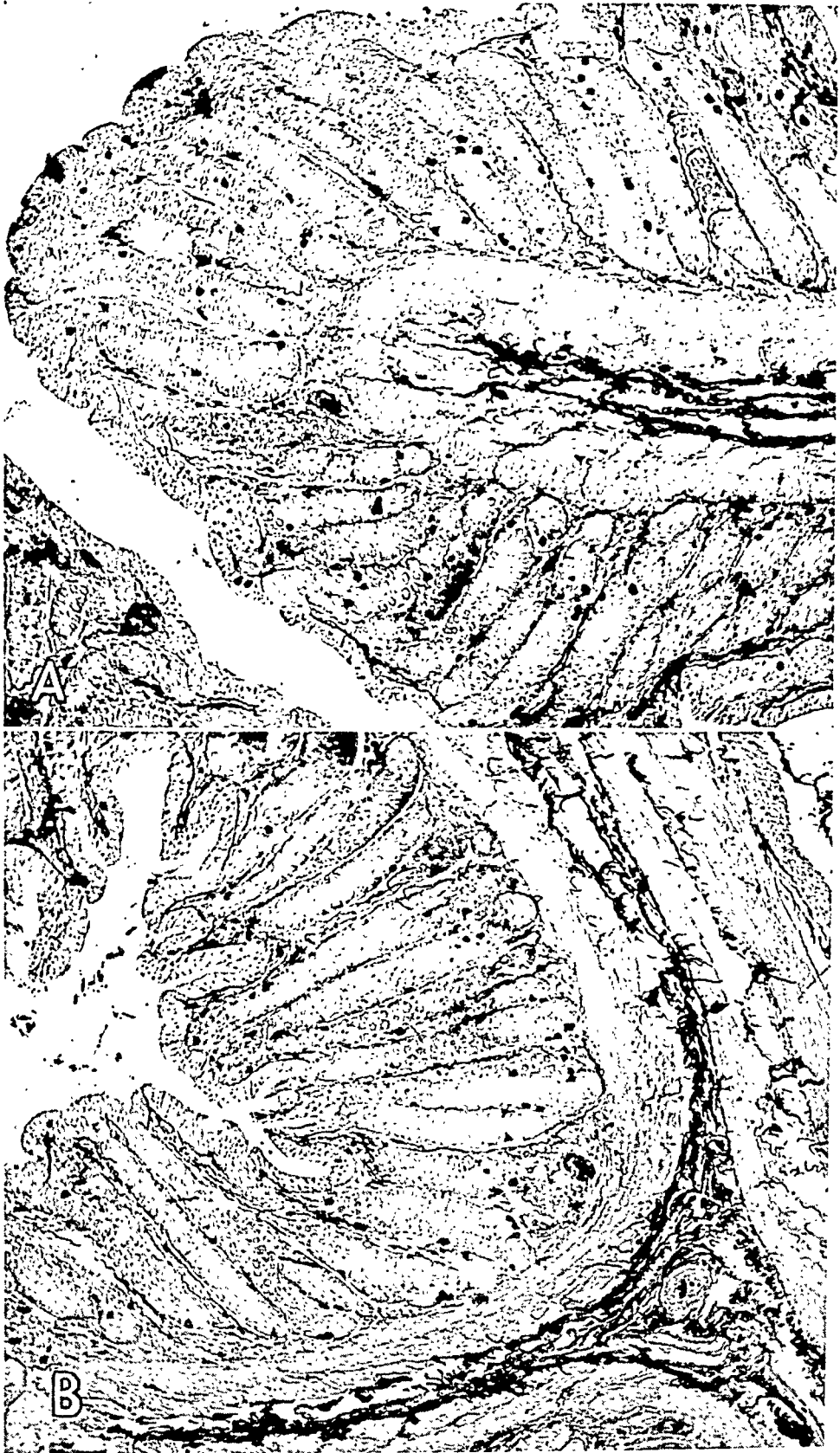


Fig. 1.—Two fields of the same section, showing argentaffin cells equally numerous in the tip and in the bottom of a fold of the sigmoid ($\times 100$).

silver-reducing property (fig. 2 *A*). These cytoplasmic and nuclear phenomena are observed repeatedly and in a vast variety of materials. With the second, or safranin-silver-water blue, method, some of the mucous cells stain a deep safranin-Van Dyke brown, some pure deep safranin, some light safranin; some take only water blue, and certain cells do not take any stain, remaining, so to speak, chromophobe (fig. 3 *C* and 11 *B*). The silver reduction retains a selectiveness similar to that obtained with the first method. These observations indicate that there is a definite interrelationship between tinctorial types of mucous content and the positive or the negative reduction shown by the corresponding cells. The first appearance and gradual increase in cytoplasmic and nuclear power to reduce silver takes place side by side with a tinctorial change of mucous content from pure safranin to mixed safranin-Van Dyke brown.

The nuclear changes just described are of considerable importance. The information furnished with the two methods applied is insufficient, however, to enable one to draw any conclusions as to the significance of the nuclear changes observed. With the third, or potassium bichromate-eosin-silver-water blue, method, some nuclei take water blue, some show affinity for eosin, and some show positive reduction of silver. It is in those mucous cells which show nuclear affinity for eosin or silver that cytoplasmic silver-reducing granules are found (fig. 11 *C*).

It appears, then, that the structure of the normal sigmoid as seen with the new methods is far from simple. There can be no doubt that under normal circumstances the mucous cell passes repeatedly through successive phases of secretory activity. As the result of slow and incomplete evacuation the fully developed mucous cell, which will be designated as cell type 1, undergoes a change in appearance and is transformed into cell type 1-a. When evacuation is rapid and complete, type 1 is transformed into a slender, darkly staining cell, or type 1-b. In both instances the cell after evacuating its contents returns to the original state or, so to speak, becomes type 1. These repeated and reversible changes represent the normal functional cycle of the fully developed mucous cell (fig. 4 *I*).

In tracing these reversible morphologic changes one sees that in some instances certain mucous cells fail to empty their content in the usual fashion. The retained mucous content acquires gradually the appearance of inspissated mucus, and its tinctorial property is changed. Instead of pure safranin, it stains safranin-orange or safranin-brown (fig. 11 *B*). The nucleus begins to take eosin and later on acquires the silver-reducing property. Side by side with this there appears in the cytoplasm a granular silver-reducing substance. The latter is found more abundantly in the basal and perinuclear zones of the cell. This cell is designated as type 1-c (fig. 2 *A*). As a further step in cytomorphosis, type 1-c is

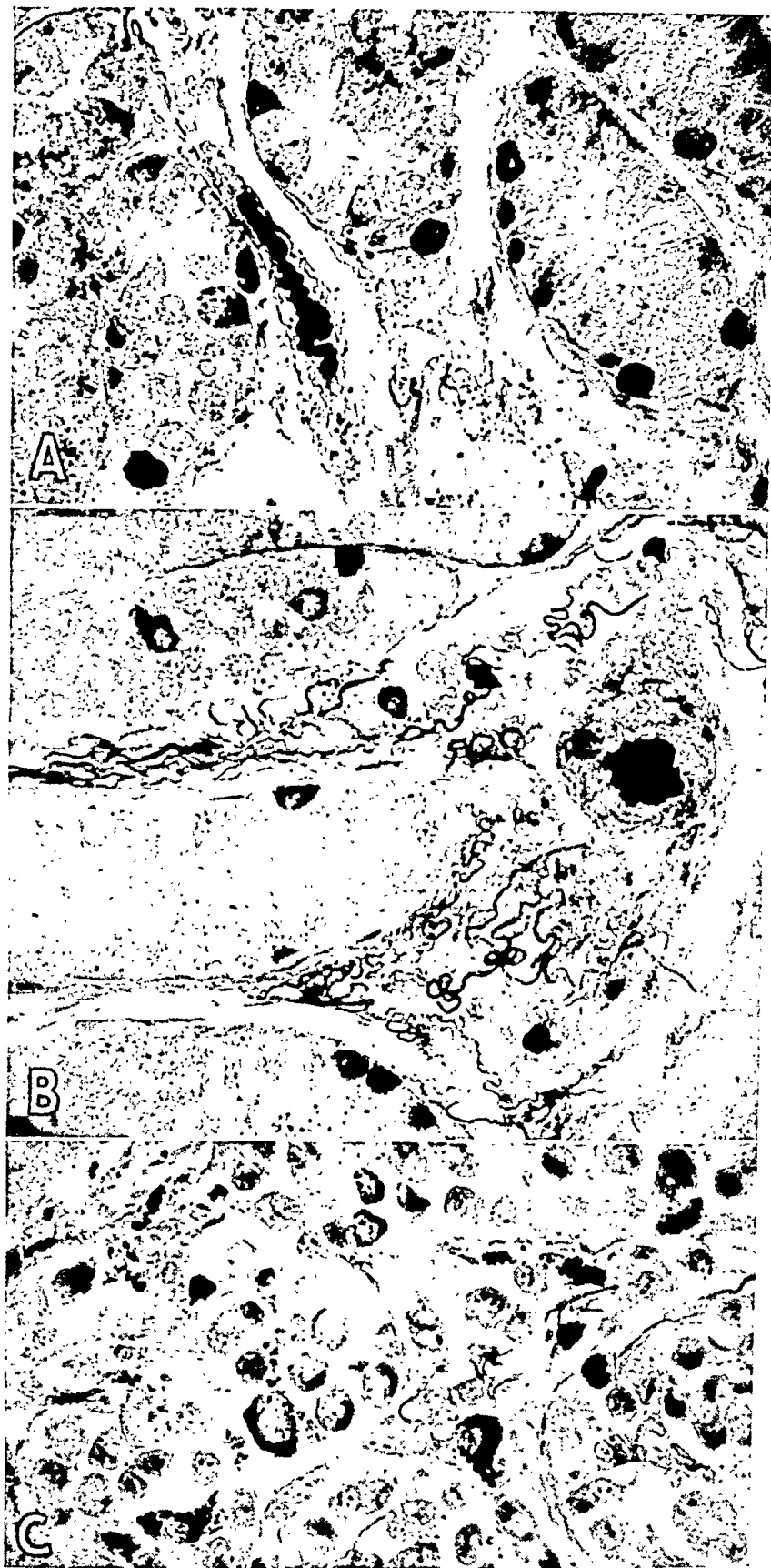


Fig. 2.—*A*, first stage, and *B*, second stage, in transformation of goblet cells of the sigmoid into argentaffin cells. In both *A* and *B* erythrocytes show selective reduction with silver. *B* shows extraglandular argentaffin cells of a mesenchymal nature; *C*, metastatic focus of gelatinous carcinoma with typical argentaffin cells ($\times 430$).

altered in the following ways: (1) It shrinks, and the cytoplasmic connection with the lumen of the intestine becomes progressively narrower; (2) it loses its property of staining with safranin; (3) its silver-reducing substance becomes more condensed and prominent; (4) the entire cell sinks gradually toward the basement membrane, and (5) the nucleus appears pushed to the basal part of the cell. It resembles now a typical argentaffin cell and is designated as type 2 (fig. 2 *A*, upper cell in central gland, and fig. 4). On further recession of type 2 toward the basement membrane, the cytoplasmic connection with the lumen of the intestine becomes narrower and more pointed, and finally the cell retracts to such an extent that connection with the lumen no longer exists. It is now discoid, the inner convexity, facing the basement membrane, being flatter and broader. The nucleus of this cell loses its affinity for eosin and shows no silver-reducing property, and its cytoplasm appears packed with condensed silver-reducing substance. This cell is designated as type 3 (figs. 2 *B* and 4). Together with this type of cell are found similar cells having a more flattened, disklike shape, the content of which instead of being black appears brown, orange-brown or yellowish brown. This fading cell is designated as type 4 (figs. 3 *B* and 4). On comparing different varieties of this type of cell, one feels warranted in concluding that the fading and gradual disappearance of the silver-reducing property begin from the periphery of the cell and that the perinuclear zone is the last to lose its power to reduce silver. No evidence of excretion or of discharge of silver-reducing substance is found, and the cell itself does not perish in the course of this peculiar metamorphosis. It appears, then, that the transformation of the cytoplasmic silver-reducing substance into a nonreducing silver substance is executed by means of some intracellular chemical process. With the loss of silver-reducing substance, a cell is formed bearing only a faint trace of water blue, which may justly be called a chromophobe cell, or type 5 (figs. 3 *C* and 4). This cell has the form of an unevenly flattened disk with its inner convexity placed in close proximity to the basement membrane. Coexistent with the chromophobe cell, a cell is seen which is similar in appearance but shows more affinity for water blue. Its upper convexity appears more rounded, and it shows a rudimentary cytoplasmic protrusion directed toward the lumen of the intestine. This cell is designated as type 6. On tracing the next step in the metamorphosis of this cell, one finds that the cytoplasmic protrusion directed toward the lumen is longer and more pointed. This cell is designated as type 7. It gradually changes its entire orientation and fixes its anteroposterior axis perpendicularly to the basement membrane. It regains its full property of staining with water blue and finally shapes itself into an elongated cell which is indistinguishable from the indifferent epithelial cell and is generally regarded as the stem cell in the formation of new



Fig. 3.—*A*, relation of the epithelial argentaffin cells to the basement membrane. *B*, further stage in transformation, with fading of argentaffinity. *C*, stage showing transformation of argentaffin cells into chromophobe cells. *D*, inverse relationship between mitoses and argentaffinity: mitoses numerous and argentaffin cells absent. (All figures from sigmoid; $\times 430$.)

mucous cells. This cell is designated as type 8. The evidence afforded by further studies indicates that the indifferent cell formed as the result of the just described cytomorphosis is able to undergo differentiation and transform itself eventually into a fully developed mucous cell. Integration of the observations just reported (figs. 4 II and 12 III), which have been verified in a large variety of materials, permits the conclusion that they represent a chain of cellular changes which are fundamentally different from the cellular changes associated with the repeated successive phases of the secretory activity of the normal mucous cell.

The consensus is that the number of mitoses found in normal organoid tissue serves as an index of cellular efficiency in replacing worn out elements. It is believed that mitoses are not found in goblet mucous cells at all or are of very rare occurrence. Mitoses are observed in the indifferent cells of the sigmoid, but their number is certainly in disproportion to the wear and tear to which the mucosa of this part is subjected continuously. In an examination of thousands of serial sections not a single mitosis is found in true goblet cells. The number and distribution of the mitoses found in the indifferent epithelial cells differ in an unusual way. In following up a long series of sections one notes that many consecutive sections may be free from mitoses and that then mitoses appear in great numbers but are seen only in particular areas of the microscopic field. The unusual feature of these particular areas is that while they are rich in mitoses they show no argentaffin cells. Furthermore, when mitoses are looked for in areas rich in argentaffin cells, they are not found. It appears, therefore, that *the number of mitoses found in the indifferent cells is in inverse relation to the number of argentaffin cells found in the same area*. Figure 3 D serves to demonstrate the described relation between the number of mitoses in the indifferent cells and the number of typical argentaffin cells found in the same field. In this microscopic field there are more than 10 mitoses with not a single argentaffin cell present. Such a peculiar relationship indicates that when cytomorphosis of cell type 1-c proceeds normally there is no call for mitoses and that when cell type 1-c fails to continue its cytomorphosis mitoses are called on to compensate for the failure (fig. 4).

Silver reduction effects obtained with formerly used methods place in the foreground only typical argentaffin cells. The older methods (Fontana-Masson, Hasegawa and others) are worthless in demonstrating the genetic relations of argentaffin cells to other elements of the mucosa, and quantitative studies made with their aid fail to offer any reasonable explanation of the quantitative discrepancies observed. As a result of this the conclusion is drawn that the so-called argentaffin cell is a special cell, *sui generis*, mysterious in origin and fundamentally different in function. When the genetic relationship between mucous

and argentaffin cells is taken into account and when closely related cells, for instance, types 1-c, 2 and 3, are counted, the total count may be as high as 40 per cent of all cells. Such extensive cytomorphosis proceeding in the absence of mitotic activity in the same area is suggestive and certainly must be of definite biologic significance. It is worthy of note that when in a particular area cells of types 1-c, 2 and 3 are present, cells of types 4, 5 and 6 are very rare. The synchronous appearance of cells closely related only genetically signifies that cytomorphosis may affect certain areas only and at the same time.

As they stand, the observations reported leave no doubt that argentaffin cells are related genetically to mucous cells. Mucous cells are known to be sensitive to a number of chemical stimuli applied directly to the mucosa. It is conceivable, then, that other aspects of relationship between these two types of cells may be revealed by local application to the mucosa of chemical stimulants which are known to be specific in their action on mucous cells. In studies on the effect of stimuli locally applied to mucous cells Pavlov⁶ and Pewsner⁷ employed silver nitrate solution, Babkin⁸ iodine dissolved in potassium iodide and Florey⁹ mustard oil diluted with olive oil. The results of their studies show that application of these noxious substances is followed by a rapid flow of mucus, with evacuation of the mucous content by the cells. In the experiments about to be reported the effect of the following substances applied locally was studied: (1) mustard oil (3 per cent in olive oil), (2) 5 per cent silver nitrate, (3) aqueous solution of iodine, (4) 5 per cent lactic acid and (5) 20 per cent magnesium sulfate. All five of these substances produce an excessive flow of mucus and yet, in spite of the severity of the injury sustained, not all mucous cells lose their content. The cells which fail to empty themselves are similar in every respect to the cell type 1-c described. Their content is stained safranin-Van Dyke brown-orange, and their cytoplasm shows the presence of granular silver-reducing substance. It appears that, compared with cell type 1, these cells remain refractory and are not influenced even by the powerful stimulative action of the chemicals applied. These cells are apparently lacking in normal response to secretory stimuli. As to cells of types 2 and 3, or typical argentaffin cells, it is found that they cannot be forced to discharge or to expel their silver-reducing content. They retain their usual intensity of silver reduction and appear undisturbed by the drastic action of the chemicals employed.

6. Pavlov, I. P.: *Le travail des glandes digestives*, translated by V. Pachon and J. Sabrazes, Paris, Masson & Cie, 1901.

7. Pewsner, M.: *Berl. klin. Wchnschr.* **44**:41, 1907.

8. Babkin, B. P., cited by Florey.⁹

9. Florey, H.: *Brit. J. Exper. Path.* **11**:348, 1930.

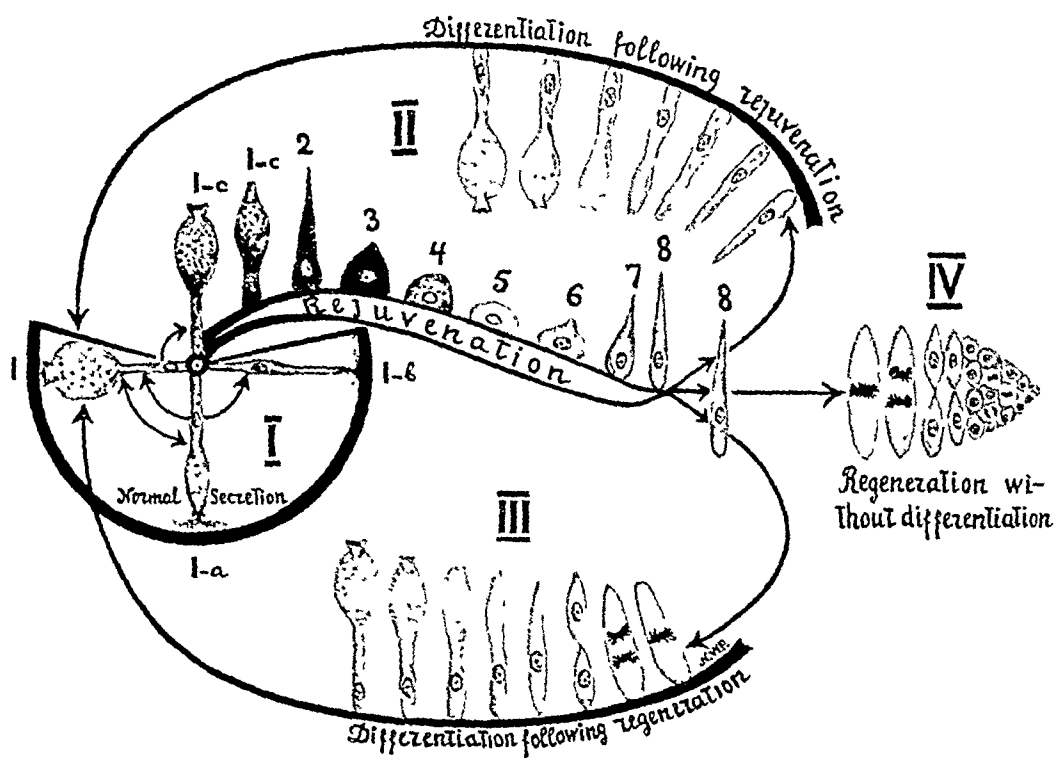


Fig. 4.—I, normal repeated successive secretory cycle of the goblet cell. II, rejuvenation cycle of a functionally exhausted goblet cell, followed by differentiation of the rejuvenated cell. III, rejuvenation cycle, followed by regeneration with consequent differentiation. IV, regeneration without consequent differentiation hyperplasia-neoplasia.

Cordier¹⁰ reported that pilocarpine applied intravenously produces discharge of argentaffin granules and that elimination takes place at the apical pole, indicating the exocrine polarity of these cells. Though this observation is reported by an investigator whose contributions to the subject of argentaffin cells are most valuable, it requires further investigation. The question of the mechanism and of the specificity of the action of pilocarpine on the mucosa of the intestines is still unsettled. From the excellent experimental work of Florey⁹ on the secretion of mucus by the colon it is clear that any influence that pilocarpine has on colonic secretion of mucus is totally different from that on glandular secretion, e. g., that of the salivary glands. To produce secretion of mucus in the colon, relatively enormous and repeated doses of pilocarpine must be injected intravenously and allowed to act over prolonged periods. Florey stated that pilocarpine applied locally to the rectum causes, after a few minutes, marked secretion of saliva, tears and bronchial mucus but an inappreciable secretion of intestinal mucus. It thus appears that claims of a specific action of pilocarpine on the intestinal mucosa lack a physiologic foundation. In the studies presented here pilocarpine was applied locally and intravenously, and in both instances argentaffin cells remained unaltered. No discharge of silver-reducing substance was shown in apical or in basal parts of the cell, and this substance was never found discharged into the lumen of the sigmoid.

The state of argentaffin cells in different phases of digestion was studied, and the results fail to show any relation of these cells to digestion. Nothing strikingly different was found in experiments with complete starvation for seventy-two hours. It may be noted, however, that in the sigmoid of an animal starved for this period the number of argentaffin cells appears to be larger. The type of cell that appears prominent numerically is type 1-c. On comparing the effect of a diet of dry oats of short duration with that of a diet of carrots and lettuce it is observed that the former leads to an increase in the number of cells of type 1-c while the latter is accompanied by a decrease in the intensity of the silver reduction effect shown by cells of types 2, 3 and 4.

In not a single instance were epithelial argentaffin cells found moving or migrating through the basement membrane in a normal colon or in the colon of an experimental animal. When sections were cut parallel to the basement membrane and close to the basal parts of the gland, the argentaffin cells sometimes created the impression of being located behind the basement membrane, and that this was an artefact is apparent when the argyrophil reticulum is shown properly (fig. 3 A).

As part of the general plan the effect of delay in fixation was investigated.

10. Cordier, R.: *Arch. de biol., Paris* 36:427, 1926.

A rabbit was kept fasting for twenty-four hours and then disposed of by air embolism. The abdomen was opened immediately, and a small segment of sigmoid was ligated on both sides, the ligation including the mesenteric blood supply. The segment was removed gently and without escape of blood from the removed segment or from the adjacent sigmoid left in. The abdomen was then closed and the animal left at room temperature. Four hours later the adjacent segment of the sigmoid was removed. In both instances the segments removed were fixed immediately in Helly's fluid and later on treated in exactly the same way. The difference in the microscopic pictures of the two segments removed revealed that the second segment failed to show in the tinctorial and impregnation results the degree of selectiveness manifested in the first specimen. Safranin staining effects remained unchanged in both segments, but the four hour delay in fixation had increased the number of cells which showed nonspecific silver reduction. It was not so much the cytoplasm as the nuclei of the cells which began to show this nonspecific reduction.

The findings indicate that metallic reduction tests are fully reliable only when they are applied to perfectly fresh tissues.

Summary.—Among the observations on the sigmoids of the 62 rabbits the following stand out as particularly important:

1. The mucous cells of the sigmoid are not identical in morphologic character and functional capacity. The nucleus and cytoplasm of a mucous cell of type 1-c shows the property of metallic reduction, and its mucous content is different tinctorially. Functionally, cells of this type are refractory and fail to respond to secretory stimuli.

2. Cells of type 1-c are not ordinary degenerating elements, and their elimination by necrobiosis or phagocytosis is not seen.

3. Argentaffin cells of types 2 and 3 are related genetically to cells of type 1-c.

4. Under normal circumstances the silver-reducing substance of argentaffin cells is never found excreted into the lumen of the intestine, and it is not demonstrated extracellularly at the base of the cell. This substance cannot be forced to leave the cell even under the influence of drastic irritants applied to the mucosa, and both local and intravenous applications of pilocarpine fail to affect these cells.

5. Argentaffin substance, probably a specific diphenol (Vialli and Erspamer¹¹), is formed in the cell in the course of its cytomorphosis and is transformed into nonreducing substance by some intracellular chemical process (probably oxidation). As a result of this, the cell is transformed into a chromophobe element which in its shape and position is identical with the argentaffin cell.

6. In the course of further stages of cytomorphosis the cell returns to the state of an indifferent epithelial cell and is then indistinguishable from the rest of the undifferentiated elements of the epithelial lining.

11. Vialli, M., and Erspamer, V.: *Ztschr. f. Zellforsch. u. mikr. Anat.* **27**:81, 1937.

7. Under normal circumstances the number of argentaffin cells found varies; they may be numerous in some sections, and in other portions of the same block they may be absent entirely.

8. Their number is not influenced by local application of the chemicals tested and is not affected in a striking way by phases of digestion, starvation for seventy-two hours or types of diet employed in these studies.

9. The finding in one area of cells belonging to the same phase of cytomorphosis indicates that their cytomorphosis takes place at the same time and affects only particular areas.

10. The number of mitoses found in indifferent cells is in inverse relation to the number of argentaffin cells found in the same area. That the two processes are not observed at the same time is rather significant. It is apparent that when cytomorphosis fails regeneration by mitoses is called on to compensate this failure by production of new cells destined to reach the same goal of functional efficiency.

Chemically speaking, it is not proper to call cells which show reduction of silver and of other metallic salts argentaffin, argentochrome or metallaffin cells. Argentaffinity simply indicates that there are cells of entodermal, mesodermal and ectodermal origin which with specially adjusted methods give positive reduction of certain silver salts. Since with use of the same methods this reaction is obtained from a variety of genetically unrelated cells it offers little in itself to make understandable its significance in individual cases. It is only when it is studied in relation to the entire course of the cell's development or activity that its significance becomes understandable. In the studies presented attention was concentrated on the genetic relation of the argentaffin cell to other epithelial cells of the lining, and the results obtained show that in the sigmoid this cell simply represents a phase in the life cycle of the mucous cell.

With each point of observation substantiated by objective evidence, it is permissible to conclude that the normal secretory cycle of mucous cells is repeatedly manifested by successive phases in the activity of the same cell. When this cell finally reaches the stage of functional exhaustion, it does not perish. It becomes refractory, loses its response to secretory stimuli and undergoes a rearrangement or cytomorphosis which proceeds through various stages of dedifferentiation, with eventual return of the cell to normal secretory activity. The cytomorphosis signifies in reality functional rejuvenation of the once exhaustive mucous cell and reflects or discloses another mysterious faculty of living matter which may be called the phenomenon of functional rejuvenation.

In the light of this new concept the problem of the histogenesis and functional role of the so-called intestinal argentaffin cell finds a different

and more logical interpretation. It is apparent that the phenomenon of functional rejuvenation is of paramount importance in restoring the exhausted mucous cell to its normal function. Ability to rejuvenate makes unnecessary continuous replacement of worn-out elements by means of regeneration, and this makes understandable the discrepancies, so far unexplainable, between the scarcity of signs of cellular regeneration and the wear and tear to which the mucosa of the sigmoid is subjected continuously. This phenomenon is governed and controlled by its own mechanism and cannot be influenced by the experimental factors tried in these studies. As the appearance and disappearance of the substance possessing the property of reducing certain metallic salts is a manifestation of intracellular chemical processes which bear no relation to elaboration of a secretory product it is easily understood why this metallic salts-reducing substance is never found outside the cells and cannot be forced to leave the cells. This concept offers a reasonable explanation of the aforementioned observations on topographic distribution and quantitative interrelations between various types of cells found in the same field. When in a particular area cells fail to rejuvenate or the rejuvenated cells (type 8) fail to differentiate, regeneration takes place; this usually takes the normal course, but occasionally it takes a neoplastic course. The whole sequence of events and the results of deviations in the process of rejuvenation are shown in figure 4.

RESULTS FROM STUDY OF THE HUMAN LARGE INTESTINE

The material comprised fresh surgical tissues showing various inflammatory processes, benign polyps and carcinoma of different types. The results of these studies indicate that the human sigmoid shows the same two types of the cell's life cycle: one manifested by successive phases of secretory activity and the other by cytomorphosis of the nature of rejuvenation. In pathologic conditions these two cycles appear to be more complicated. The reaction on the part of the mesenchymal elements is the chief source of confusion. Degenerative processes come to the foreground, and a great number of cells of type 1-c fail to rejuvenate. Failing to regenerate, they degenerate. Their content escapes and is taken by phagocytes.

The benign tumors are represented in these studies by pedunculated polyps with the structure of well differentiated adenoma. This differentiation is manifested not only by the appearance of a glandular pattern but also by the demonstration of (*a*) the normal secretory cycle and (*b*) the rejuvenation cycle of the functionally exhausted mucous cell. There is no doubt that accumulation of mucous secretion in the closed interglandular spaces of polyps raises the pressure, causes trophic disturbances and is responsible for interference with the normal course of both cycles.

From comparative studies it is apparent that the capacity for growth in benign polyps is restricted not only by the extent of histologic perfection in glandular differentiation but even more by the ability of newly formed and fully developed mucous cells to rejuvenate normally. This conclusion appears to be warranted by the observation that new epithelial proliferation, as evidenced by regeneration through mitosis, is strikingly insignificant.

The different types of carcinoma are considered according to the classification of Ewing.¹² Adenoma destruens shows a variety of structures, ranging from completely preserved normal features of the glands to carcinoma growing in disorderly fashion, without any glandular differentiation. In each case of carcinoma control studies are made from segments of the intestine above and below the carcinomatous obstruction. Sections from adenomas show that in some parts of the growth both cycles proceed equally well. Fully developed mucous cells (type 1) contain normal-appearing, safranin-stainable mucus, and the cycle of rejuvenation is evidenced by the presence of argentaffin cells. A number of cells of type 1-c appear to be degenerating and, as free cells, are seen in the interglandular spaces. This is associated with local accumulation of phagocytes. In sections represented by disorderly growing cells, with no evidence of any glandular arrangement, nothing is found to indicate a manifestation of the two cycles shown by the mucous cell. Degeneration and mitotic activity here dominate the entire picture.

The particular feature of typical carcinoma is that no cells are shown containing safranin-stainable mucus or any signs of rejuvenation. Gelatinous carcinoma differs in many ways from the tumors discussed. In some areas it shows an alveolar structure consisting of poorly differentiated cells free from safranin-staining content. It is apparent that these cells possess the property of further differentiation, and, as a result of this, mucous cells containing safranin-stainable mucus are formed. These act as true secretory cells, profusely discharging their content into the free pericellular spaces. With no normal secretory stimuli on hand and lacking an outlet for the secretory product, each becomes gradually distended and ballooned and the nucleus is compressed into a signet ring form. Even under these abnormal circumstances the cells are capable of completing and repeating their cycle of secretion many times before they reach the stage of exhaustion. Instead of perishing, some of these exhausted cells undergo complete rejuvenation cytomorphosis, with transformation of the exhausted cells into typical argentaffin cells, as shown in figure 2C. The significant feature of this photomicrograph is that it represents a section of infiltrating growth in the serous coat. With both cycles available there is no need for

12. Ewing, J.: *Neoplastic Diseases: A Textbook on Tumors*, Philadelphia, W. B. Saunders Company, 1934.

continuous regeneration, and it becomes understandable why mitotic activity is least prominent in gelatinous carcinoma. This explains also the tremendous capacity of the cells of gelatinous carcinoma to produce mucus, an excess of which is a specific feature of this type of carcinoma. These studies indicate that the index of malignancy cannot be evaluated on the basis of perfection in the degree of glandular differentiation. This index is reflected or better shown by the demonstration of an ability or an inability of the cells of a neoplasm to function (in the sense of successive cycles of secretion) and to rejuvenate (in the sense of rejuvenation of functionally exhausted cells). Demonstration of these two phenomena is impossible with the hematoxylin-eosin staining which is in use in present day methods of grading the malignancy of epithelial neoplasms.

As already mentioned, in each case of tumor growth a segment from above and one from below the obstruction were taken for examination. The comparison of these sections is very instructive. In many instances the segment from above the obstruction shows changes which are not observed in the segment below it. Above the obstruction cells of type 1-c are very numerous, while the argentaffin cells (types 3 and 4) are rare. This indicates that the functionally exhausted cells are unable to complete the normal cycle of rejuvenation under circumstances of continuous irritation and stasis. As a result of this the endogenous product of their gradual disintegration is taken up by macrophages, which are seen at first in the periglandular zone and later on in the deep part of the submucosa, and the content of such macrophages is browned or blackened by the silver nitrate methods employed. This explains why melanosis may be limited to a tiny area or to a patch, the neighboring mucosa being devoid of pigment. It is difficult to imagine that absorption from the intestine of some exogenous product could create such peculiarities in topographic distribution of melanosis. Below the obstruction rejuvenation proceeds uninterruptedly, and melanosis is not observed. In later life rejuvenation proceeds with greater difficulty, and it is not a coincidence that melanosis is observed most commonly in old age. Since the pigment found above the obstruction fails to give the prussian blue reaction it cannot be of hematogenous origin. These studies are in agreement with those by Stewart and Hickman,¹³ but they offer a different explanation as to the cause of melanosis coli.

RESULTS FROM STUDY OF THE APPENDIX OF THE RABBIT

The appendix of a full grown young rabbit which had been kept fasting for twenty-four hours was considered as the normal control. An additional method employed which appeared to be of value is as

13. Stewart, M. J., and Hickman, E. M.: *J. Path. & Bact.* **34**:61, 1931.

follows: Sections are prepared in the usual way, treated with hydrazine hydrate-water blue reducer for ten minutes, rinsed quickly with distilled water, treated with a solution of gold chloride (1:500) containing 0.5 per cent acetic acid for ten minutes, rinsed in water, dehydrated with alcohols and mounted in balsam. The studies included the influence of (1) fasting for seventy-two hours, (2) phases of digestion, (3) various diets and (4) local application of the substances employed in the sigmoid.

The results of studies of the sections show that (1) the type of epithelial lining is not the same in different areas; (2) the interrelation between the glandular layer and the underlying lymphoid tissue follows a definite pattern, and (3) the structure of the lymphoid tissue is different from that of the ordinary peripheral lymph node. The lymphoid tissue is represented by a continuous thick pad which separates the glandular layer from the muscular coat entirely. The surface of the lymphoid pad shows numerous conical papillary projections directed toward the lumen. In a quiescent and moderately relaxed organ these projections are 0.6 to 1.2 mm. high and 0.2 to 0.6 mm. wide at the base. Each conical formation projects into a domelike space, the roof of the dome being formed by the glandular layer of the mucosa (fig. 5*A*). This topographic arrangement resembles somewhat the interrelation between the pyramid and the minor calyx of the kidney. The top of the roof is perforated with a glandular passage which contains a number of lateral glandular pouches. The glandular layer of the roof facing the free space of the dome shows numerous goblet cells and argentaffin cells but relatively few lymphocytes. The epithelial lining covering the conical lymphoid projections shows very rare mucous cells, equally rare argentaffin cells and very numerous lymphoid elements (figs. 5*A* and *B* and 11*C*). Whenever found, mucous cells show two cycles which are similar in every way to those observed in the mucous cells of the sigmoid. Similar results are also observed in animals under experimental conditions. Epithelial argentaffin cells are not found migrating through the basement membrane, and they cannot be forced to part with their argentaffin content under the influence of pilocarpine or drastic stimulants applied locally.

A difficult problem connected with studies of the appendix is offered by the structural peculiarities of the lymphoid tissue. This tissue shows both cortical and medullary substance, but the latter cannot be compared to the medullary substance of the peripheral lymph nodes. Mitoses are disseminated all over the lymphoid tissue, although they appear more numerous in the cortex. Both the cortex and the medullary substance are furnished with argyrophil reticulum, this being more prominent in the cortex. The medullary substance has its own vascular capillary plexus, which is not as rich as that in the cortex. The methods employed

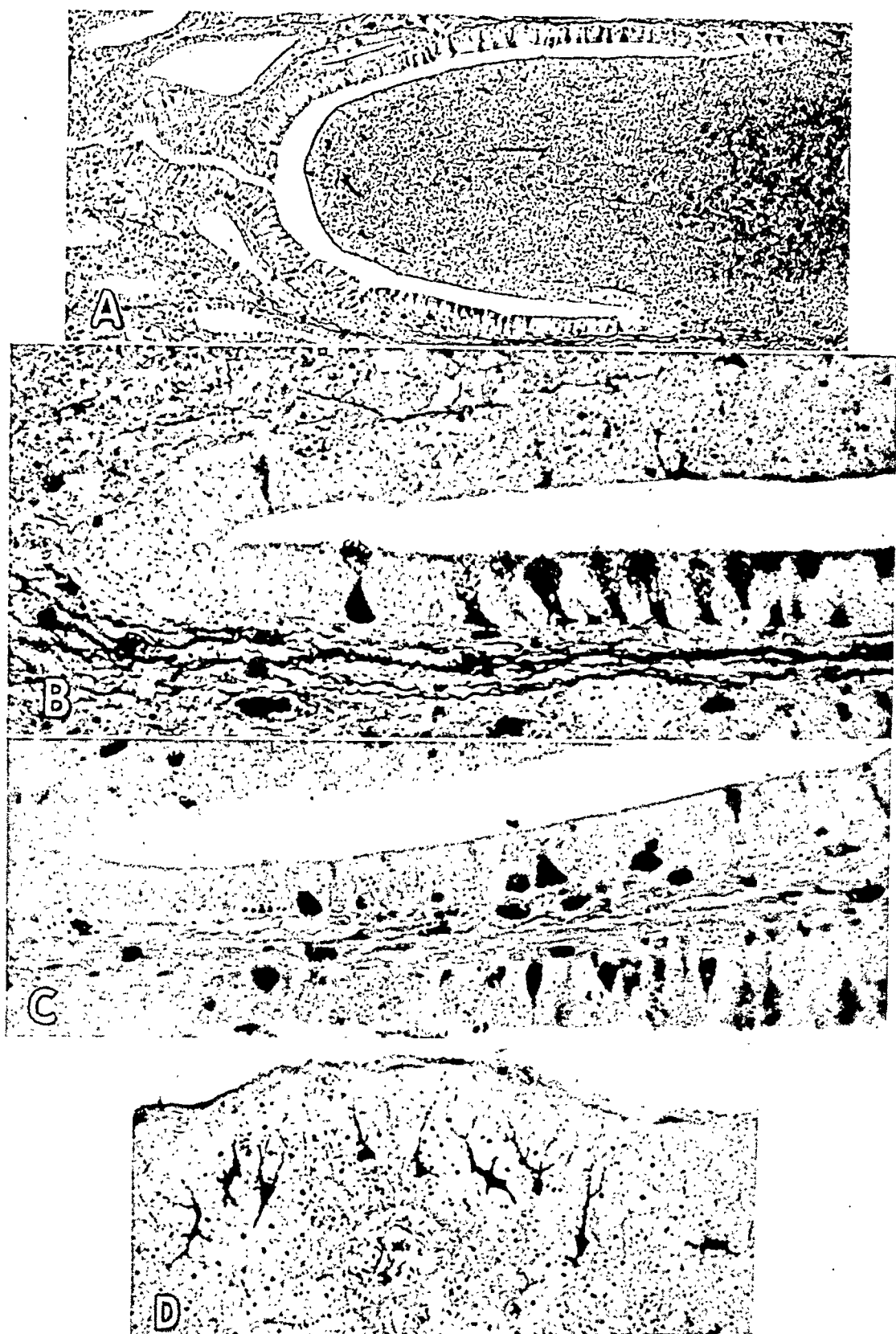


Fig. 5.—*A*, lymphoid projection in appendix showing difference in type of epithelial lining ($\times 100$). *B*, first stage in transformation of goblet cells of appendix into argentaffin cells, showing difference in epithelial lining of Lieberkühn's gland and lymphoid projection. *C*, second stage in transformation. *D*, intraepidermal cells of Langerhans ($\times 430$).

are helpful in differentiating various mesenchymal elements present in sections. Histiocytes are especially well shown. In certain phases of their activity these cells resemble closely the microglia cells which were demonstrated with the silver carbonate method by del Rio Hortega and Jimenez de Asua¹⁴ in tumors, tubercles, hepatic lesions, the normal human kidney and in lymph follicles. The advantage of the methods used in the present work over the silver carbonate method is that they (especially method 3) reveal a definite range in tinctorial and impregnation differences which makes possible differentiation of various phases of the life and activity of the histiocyte (figs. 5 *C* and *D* and 11 *C*).

On treating sections with method 1 there are seen in the cortex and sometimes in the medullary substance peculiar looking cells, scattered singly or in groups. When seen in groups, some of them take water blue and some show silver reduction of a type differing from that shown by the argentaffin cells of Lieberkühn's glands. These cells are more numerous in the cortex (fig. 6 *B*), although quite often they are found in the medullary substance (fig. 6 *A*). When found in groups, they appear as giant cells with a voluminous crown of silver-reducing cells and small round cells taking water blue and containing a small confluent chromatin mass (fig. 6 *C*, right side). The peripheral cells appear to have an intimate connection with the pale cells scattered in the vicinity, which differ morphologically from the rest of the cellular elements. These formations are quite large, and sometimes one such formation is seen uninterruptedly in thirty consecutive sections 7 microns in thickness. At one level of sectioning cells are light blue, while at other levels they are light or dark brown. The content of the central cavity of the giant cell formation is either colorless or yellowish brown, or it consists of brown droplets or dark brown granules. In certain instances these formations show their own argyrophil reticulum. In tracing their structure in serial sections it is found that some of them have at one or another level of sectioning the unmistakable form of a tubule surrounded with fine reticulum (fig. 6 *C*, left side). In further sections of the same series the tubule gradually changes its shape; it becomes disfigured, and finally the whole structure acquires the appearance of multicellular formations arranged without any particular order. There can be no doubt that these formations are epithelial in nature and that they are endowed with some property of glandular differentiation. Topographic demonstration of these formations is best achieved with the gold chloride method described in the beginning of this section. With this method they stand up as black or dark brown cells on a light blue

14. del Rio Hortega, P., and Jimenez de Asua, E.: *Arch. cardiol. y hemat.* 2:161, 1921. Jimenez de Asua, F.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* 109: 354, 1927.

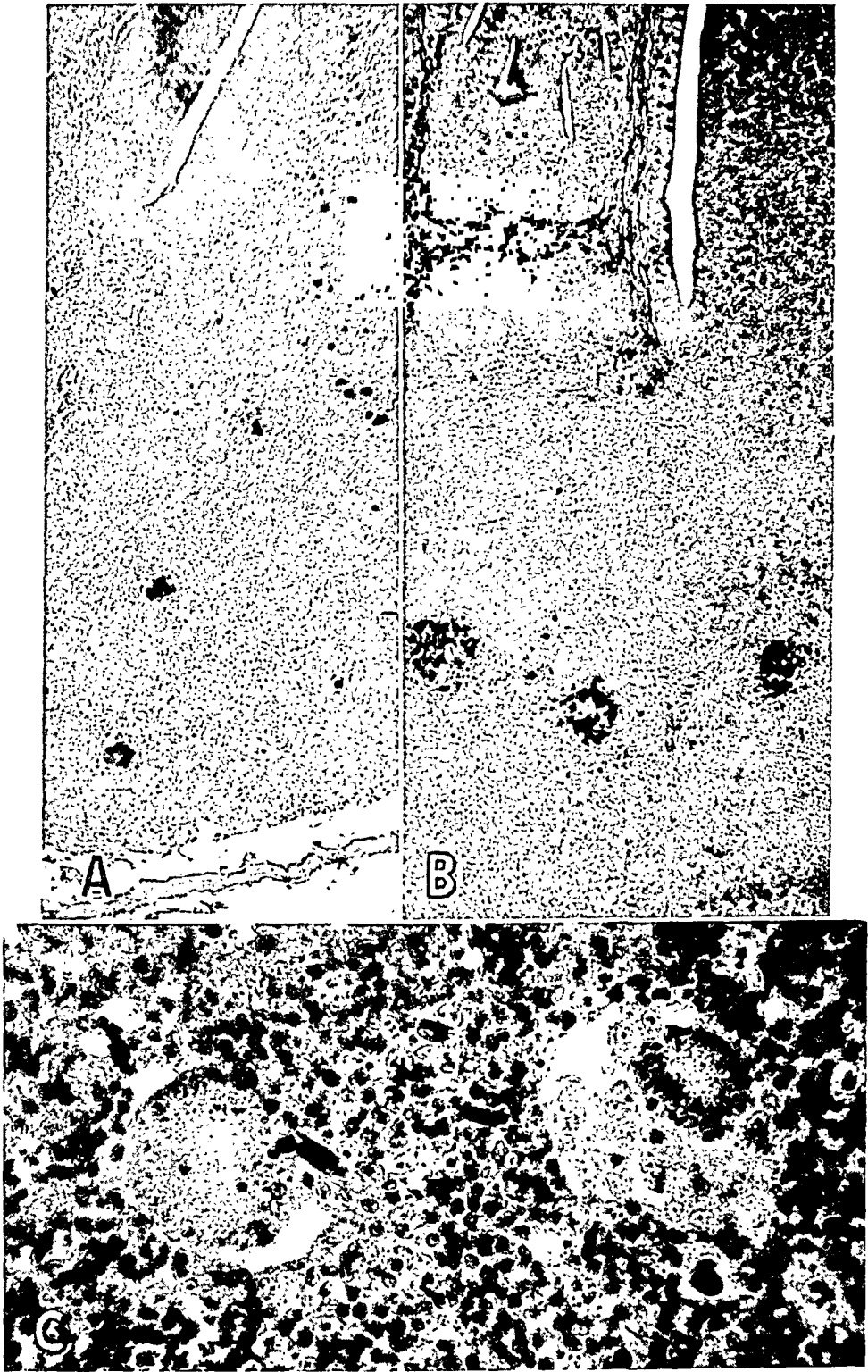


Fig. 6.—*A* and *B*, epithelial plaques in the cortical and medullary substance of the lymphoid tissue of the appendix ($\times 100$). *C*, epithelial giant cell formations with tubular differentiation in the medullary substance of the lymphoid tissue of the appendix ($\times 430$).

background (fig. 6 *A* and *B*). It is interesting that with this method the only content of some of these cells and the content of certain goblet cells of Lieberkühn's glands give gold reduction, while the mesenchymal cells show no signs of gold reduction. In some instances gold-reducing cells are not found; instead, there are seen finely granular cells of a signet ring appearance. In conclusion it may be said that in the rabbit the lymphoid tissue of the appendix is a lymphoepithelial tissue and resembles in every way the structure of the lymphoepithelial tissue of the bursa of Fabricius, which has been investigated and described with unsurpassed thoroughness by Jolly.¹⁵ With method 3, the epithelial anlage is shown much more clearly than with the hematoxylin-eosin-azure stain used by me¹⁶ in studies of the histogenesis of the thymus. With their location excluding external secretion, these cells with limited power of glandular differentiation pursue their own mysterious course of life. No cycle of rejuvenation similar to that observed in the goblet cells is disclosed. Only on rare occasions are cells found in the cortex which resemble the argentaffin cells of Lieberkühn's glands. In areas where the cellular and extracellular metallic salts-reducing content is abundant the phagocytes appear also in increased numbers, and the pigmentosis or melanosis of the lymphoid tissue in such instances is undoubtedly due to excessive accumulation and phagocytosis of a substance produced by the epithelial cells present in the lymphoid tissue. This is corroborated also by the negative results of the berlin blue reaction.

Summary.—From the observations in the appendixes of the 40 rabbits examined it is apparent that epithelial cells of the mucosa, which possess the property of reducing certain metallic salts, in their origin, sequence of intracellular changes and functional significance are identical with similar cells of the sigmoid. Differences in the morphologic appearance of argentaffin cells found in the appendix are closely related to differences in the appearance of mucous cells found in corresponding parts of the mucosa. For instance, in the epithelial coat of outer parts of the glands the mucous cells are low and small and here, too, only small argentaffin cells are found. The most convincing evidence of genetic interrelations between mucous and argentaffin cells is shown by the epithelial lining covering the conical lymphoid projections directed toward the lumen of the appendix. In this epithelial lining the goblet cells are very rare and here, again, the argentaffin cells are equally rare. As in the sigmoid, the pigmentosis or melanosis of the appendix is precipitated by interference with, or interruption of, the normal cycle of

15. Jolly, J.: *Arch. d'anat. micr.* **16**:362, 1914.

16. Popoff, N. W.: *Proc. Soc. Exper. Biol. & Med.* **24**:148, 1926; *Arch. f. exper. Zellforsch.* **4**:395, 1927.

rejuvenation of functionally exhausted mucous cells, and since the mucosa of the appendix is represented solely by one functional type of cell (mucous cell) it is understandable why the process of melanosis observed is most commonly found in the appendix. The finding of lymphoepithelial tissue in the appendix opens a new approach to studies of the physiologic and pathologic aspects of this organ. Figures 5 *B* and *C* and 11 *C* demonstrate nuclear and cytoplasmic changes associated with rejuvenation. Figures 5 *B* and 11 *C* correspond to an early stage and figure 5 *C* to a later stage of transformation of the functionally exhausted goblet cells into argentaffin cells.

RESULTS FROM STUDY OF THE HUMAN APPENDIX

The material examined included inflammatory conditions of acute, subacute and chronic types and a number of neoplastic processes (benign polyps and carcinoids). As stated previously, delay in fixation, even for four hours, interferes with the selectiveness of reduction methods, and for this reason only fresh surgical material, fixed immediately, was used in this work. Almost all appendixes removed surgically are pathologic in one way or another, and this excludes the possibility of making control studies on perfectly fresh normal organs removed from persons of different ages. In studies of lymphoid, and especially of lymphoepithelial, tissues the age of the animal, the state of nutrition and other factors concomitant with infection and toxemia are of great importance. Generally speaking, it is unreasonable to depend on clinical material in studies which pertain to fundamental problems of histophysiologic nature. With no way of knowing the nature of the inciting factor and the length of time it had been at work, it is difficult to tabulate qualitative and quantitative changes in an orderly manner. As far as general conclusions are concerned, it may be said that observed under pathologic conditions the essentials in the cycle of rejuvenation remain the same as when observed in normal mucosa. There is a great deal of individual variation, but the fundamental significance of the phenomenon of rejuvenation is unchanged. In cases in which appendical or fecal stasis is a predominant feature there is increase in the number of cells of type 1-c. With the safranin-silver-water blue method the mucous content of these cells stains differently, and their cytoplasm is filled with silver-reducing granules. It is apparent that because of stagnation and abnormalities in stimulation the mucous cells are unable to continue the normal cycle of secretion for their usual length of time. Functioning under such abnormal circumstances, they are unable to evacuate their content in normal fashion, and at the same time the exhausted cells, with unevacuated content, are not given proper conditions for completing the normal cycle of rejuvenation. This explains

the scarcity of argentaffin cells found and makes understandable why under such circumstances only cells of type 1-c and active phagocytes containing silver-reducing substance are prominent in the picture of appendical stasis.

As to the structure of lymphoid tissue, it may be said that pathologic material is unreliable for studies of this subject. In the majority of cases examined, such tissue is either absent (owing to complete atrophy), or it is distorted and altered to such an extent that little is left for dependable deduction. A few appendixes removed from children in cases of mistaken diagnosis of appendicitis were examined, and some of these showed well preserved lymphoid tissue. Studies of this material leave no doubt that human lymphoid tissue contains entodermal elements similar to those found in the appendix of the rabbit. The entodermal elements are observed most commonly in the cortex, while in the medullary substance they are discernible with great difficulty. The glandular differentiation observed in the lymphoepithelial tissue of the rabbit is not found in the human appendix. Attempts to demonstrate entodermal constituents of lymphoepithelial tissue with ordinary hematoxylin-eosin staining are futile, and in this work these elements are considered as entodermal only when corroborative evidence is offered by application of all four of the methods employed in studies of the lymphoepithelial tissue of the appendix of the rabbit.

A few words need to be said concerning the observations on neoplastic processes of the human appendix. A case of benign pedunculated polyp seated in the distal part of the organ (2 cm. from the tip) deserves particular attention. On comparing sections from the proximal and distal ends of the appendix with those from the polyp, one finds the rejuvenation cytomorphosis in all of the three sections examined. With an unobstructed outlet for mucous secretion in the proximal part of the appendix the lumens of the glands of Lieberkühn are free from stainable matter while the majority of the glandular cavities in the polyp are literally choked with mucus and brownish granular silver-reducing substance. Such areas appear to be invaded with a great number of active phagocytes. The epithelial lining is represented chiefly by cells of type 1-c, argentaffin cells being scarce. There are present, however, areas in the polyp which are indistinguishable from the mucosa of the appendix. As in the mucosa of the appendix, they show the usual goblet cells, argentaffin cells and cells of Paneth, and their glandular spaces are free from stagnant mucus and silver-reducing substance (fig. 11 *A*). In other words, the whole picture indicates that in such areas the process of rejuvenation pursues its course the same as in normal mucosa. Mitoses in polypous growths are very rare. It appears, then, that the life of such highly differentiated pedunculated growths is perpetuated solely by normal repetition of the secretory cycle followed by the uninterrupted cycle of rejuvenation.

There is a great deal of disagreement as to the origin of carcinoid tumors of the appendix. These tumors are specific in their topography and structure, and their cellular constituents show quite often the property of silver and chrome reduction. Masson¹⁷ expressed the belief that migration of epithelial argentaffin cells occurs and that carcinoid tumors are formed as the result of budding at the tip of Lieberkühn's gland. A number of investigators failed, however, to demonstrate the phenomenon of budding described by Masson. The majority of carcinoids are removed at a time when little is left of the normal structure of the appendix: The lymphoid tissue is gone, the entire submucosa is replaced with tumor cells, and under such circumstances it is rather difficult to reconstruct a true picture of the changes which took place at the very beginning of the growth. In many publications it is stated that tumor cells are found immediately beneath the lining cells, and this is taken as evidence that the tumor has its source in the crypt of Lieberkühn. If a tumor growth originates in the Lieberkühn gland, it is strange that no one ever published a case of carcinoid having its seat in this gland and forming an everted growth. A great number of benign and malignant tumors definitely begin in glands of Lieberkühn. They grow as everted tumors, and though they may show the presence of argentaffin cells they are fundamentally different in behavior and structure from carcinoids. As a rule carcinoids grow as inverted tumors or, speaking more exactly, they grow beneath the glandular lining. The close proximity, however, of the protruding growth to the glandular lining cannot be considered as convincing evidence that the tumor originates from the lining of Lieberkühn's gland. Forbus¹⁸ reported 6 cases of carcinoid, case 6 of his series being most interesting. The tumor was found in a patient having generalized miliary tuberculosis. It was located in the submucosa of the ileum, the mucosa being well preserved. Though no direct connection was found between it and the glands of the mucosa, the tumor cells extended close to the zone of the deepest crypts. No mention is made of the appearance of the lymphoid tissue in the region of the tumor or in Peyer's patches, the structure of which—as will be shown later—is not that of ordinary lymphoid tissue. In a majority of the reports of cases in the literature nothing is said about the lymphoid structure in the region of the tumors. Until the question of the initial relation of these tumors to the lymphoepithelial structures is given due consideration, nothing definite can be said as to the exact origin of carcinoids. In almost all the cases studied in the present work there was a failure to show any remnants of original lymphoid tissue, and attempts to find the source or seat of the primary growth seemed fruitless and futile. In only 1 of 6 cases examined were remnants found

17. Masson, P.: *Am. J. Path.* **4**:181, 1928.

18. Forbus, W. D.: *Bull. Johns Hopkins Hosp.* **37**:130, 1925.

which could be considered as original lymphoid tissue, and the intimate relation between the epithelial and the lymphoid tissue was found to be identical with that observed in the lymphoepithelial tissue of the appendix of the rabbit. It is worthy of note, too, that when a solid cord of carcinoid growth shows incipient phases of glandular differentiation these primitive glandular structures resemble in every way similar structures found in the lymphoepithelial tissue of the rabbit's appendix. In a case of carcinoid examined the epithelial structures showed complete glandular differentiation. Here the tubules were surrounded with argyrophil reticulum in the form of basement membrane, and the epithelial lining was furnished with terminal bars which were shown with unusual clarity by the silver methods employed in this work. No objective evidence was found to indicate that the argentaffinity shown quite often by carcinoid cells signifies degeneration, and it may be assumed that such argentaffinity is related to the phenomenon of rejuvenation demonstrated in epithelial cells of Lieberkühn's glands. Absence of mitotic activity and the low grade of malignancy of these slow-growing tumors serve as an indirect argument in support of such a supposition. If this supposition is correct, then, applying a functional term, the carcinoid or argentaffin tumor should be called a rejuvenocytoma of the intestinal tract.

RESULTS OF STUDY OF THE SMALL INTESTINE OF THE RABBIT

The small intestine was the main object of my work on the terminal vascular system, and this offered an opportunity for studying argentaffin cells in a great variety of materials. In the series now being reported 50 rabbits were used. The presence of the valves of Kerkring and of villi contribute enormously to an increase of surface mucosa which physiologically and histologically differs from the mucosa of the colon. The epithelial lining is represented by the following types of cells: simple columnar cells with striated cuticular border, goblet and argentaffin cells, and Paneth cells. Lymphoid tissue is represented by solitary follicles scattered all over the intestine but more numerous in the distal part of the small intestine. Aggregated follicles, or patches of Peyer, occur as a rule in the ileum, and only very seldom are they seen in the rest of the small intestine.

With a genetic interrelationship between mucous and argentaffin cells in the colon and appendix firmly established, the entire problem of this research resolves itself into the question of whether a similar interrelationship exists in the small intestine. Here the number of mucous cells is much smaller than in the colon and appendix, and on comparing different parts of the small intestine one finds that they are more numerous in the distal part of the ileum and in the duodenal

papilla close to the ampulla Vateri. Placed between high columnar cells, the goblet mucous cells of the small intestine are smaller and taller than those in the colon and usually are scattered singly throughout the epithelial lining. As do those in the colon, they show two cycles: one manifested by successive repetitions of the process of normal secretion and the other by cytomorphosis associated with rejuvenation of refractory and functionally exhausted mucous cells and eventual return of the rejuvenated cells to normal secretion (fig. 12 *II*). Since the mucous cells are less numerous and are scattered singly, the argentaffin cells are also found to be less numerous and scattered in the same way. In many instances cells of type 1-c retain the shape of goblet cells and stand out in distinct contrast to the surrounding columnar cells, which take only water blue. In the small intestine mucous cells are smaller and taller and argentaffin cells appear correspondingly more delicate and much more elongated than in the colon. The cytoplasmic continuations directed toward the lumen of the intestine are seen with unusual clarity, this variety of cells being much more predominant here than in the colon. In parts of the small intestine which are rich in mucous cells the argentaffin cells are observed in correspondingly greater numbers. This close quantitative interrelationship is particularly well shown in the duodenal papilla close to the ampulla Vateri. Judging by the number of argentaffin cells found in this highly important physiologic region, one decides that here rejuvenation compensates wear and tear with the utmost vigor. In not a single instance are argentaffin cells found migrating through the basement membrane. The argentaffin cells cannot be forced to part with their silver-reducing substance under the local effect of the following substances: epinephrine, physostigmine, histamine, pilocarpine, atropine, sodium nitrite, benzene benzoate, 75 per cent alcohol, mustard oil, aqueous solution of iodine, silver nitrate, palladium chloride, lactic acid or 20 per cent magnesium sulfate. The intravenous application of pilocarpine also fails to influence the content of argentaffin cells. The experiments demonstrating these facts were performed with the technic employed in the studies of the colon and appendix. The observations on the effects of starvation for seventy-two hours, of different types of diet and of various phases of digestion show no striking difference from the results obtained in the colon and appendix. In a great number of cases the small branch of the mesenteric artery supplying the segment under experimentation was visualized by an injection of india ink in a live animal, but no particular topographic relation of the argentaffin cells to the blood vessels was disclosed. The structure of Peyer's patches is that of lymphoepithelial tissue. Entodermal cells are found here, scattered singly or in multicellular formations, which are easily discernible with the four methods employed in

studies of lymphoepithelial tissue of the appendix. Multicellular epithelial plasmodial masses quite often show signs of glandular differentiation with formation of abortive tubules furnished with argyrophil reticulum. It is with great rarity that pigmentosis (melanosis) of the small intestine is found. Compared with those in the colon, mucous cells in the small intestine are much less numerous and here, too, lymphoepithelial tissue is found only in the distal part of the ileum (patches of Peyer) and is more insignificant in amount than in either the colon or the appendix. Scattered singly here and there, the mucous cells, even in cases of disturbance with rejuvenation, are unable to precipitate a local phagocytic reaction to the extent observed in the colon and appendix. Here, also, the motor activity of the villi and the absence of lateral pockets in Lieberkühn's glands prevent stagnation of products which are formed as the result of interruption of the rejuvenation cycle and which act as the inciting factor in mobilizing local phagocytic defense. These anatomic peculiarities shown by the small intestine are sufficient to explain why the pigmentosis or melanosis so commonly found in the colon and appendix is hardly ever observed in the small intestine.

CELLS OF PANETH

In a review of the literature no reference was found to positive impregnation of Paneth cells with silver methods. In an article on cells of Paneth, published in 1937, Hertzog¹⁹ said that Paneth granules are not stained by silver salts. Mols²⁰ in studies on Paneth cells employed, in addition to other methods, the methods of Castro, Golgi and Cajal, but he made no reference to the effect of metallic salts on the granules of Paneth cells. No clearcut demonstration of secretory matter produced by cells of Paneth free in the cul-de-sac of a gland is found in the available literature. Mols²⁰ stated that he was able to see in the glandular cul-de-sac granules and filaments stainable by Mann's method. His main arguments, however, are more indirect, and his drawings are not clear enough. Maximow and Bloom²¹ expressed the opinion that a discharge of granules into the lumen is rarely seen except under the influence of pilocarpine. Hertzog¹⁹ stated that no granules were ever seen outside of the cell in the lumen of the crypt.

The results obtained with the methods used in the present work show (1) that the granules of Paneth cells are impregnable with silver (fig. 7 A) and (2) that the same silver-reducing product of secretion is demonstrable with utmost clarity in the lumens of the glands without any

19. Hertzog, A.: *Am. J. Path.* **13**:351, 1937.

20. Mols, G.: *Arch. de biol., Paris* **40**:111, 1930.

21. Maximow, A., and Bloom, W.: *A Textbook of Histology*, Philadelphia, W. B. Saunders Company, 1930.

application of pilocarpine. A photomicrograph (fig. 7 *B*) shows Paneth cells in a state of active secretion, and in both left and right lower corners are seen uninterrupted connections of secreting Paneth cells

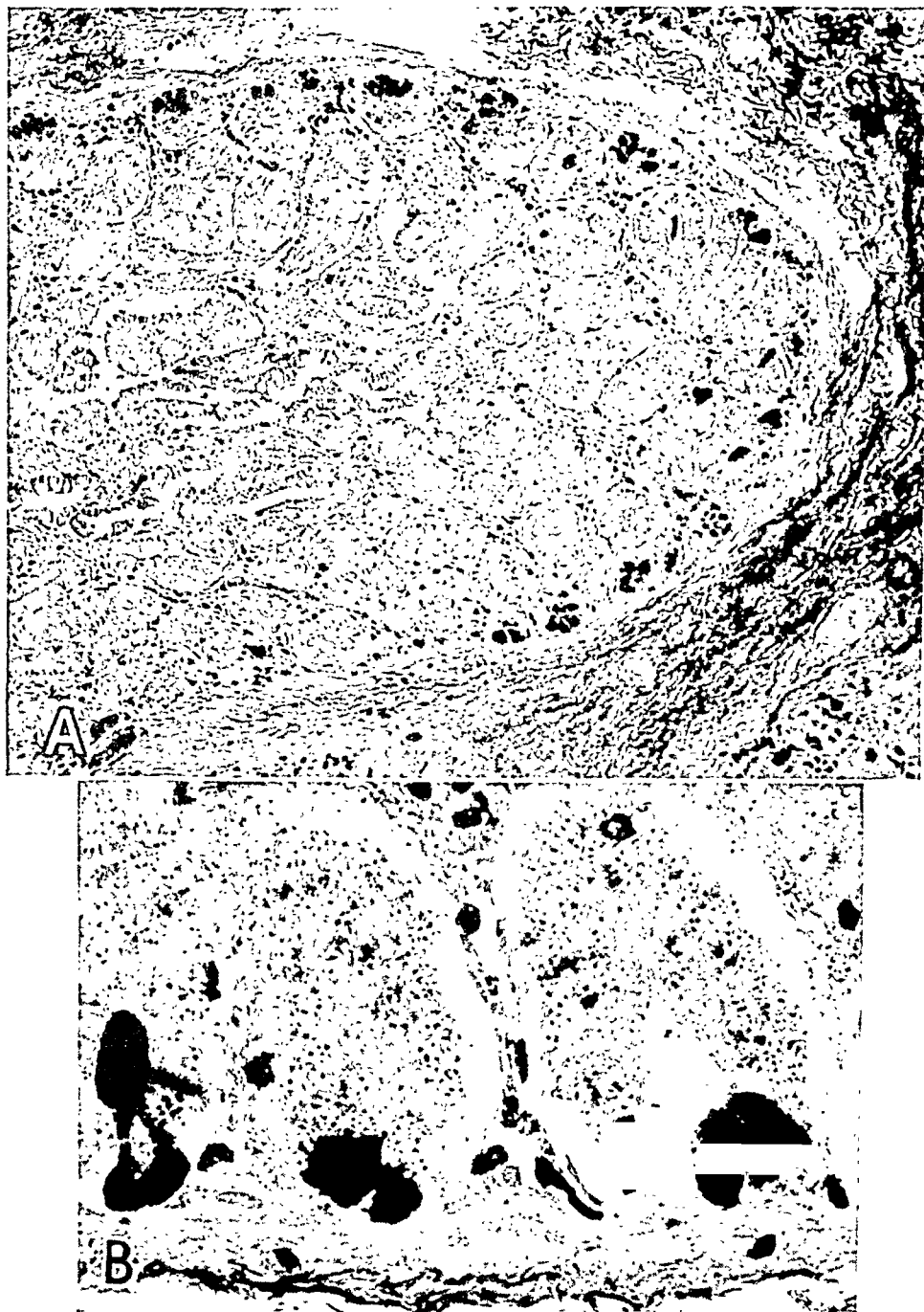


Fig. 7.—*A*, human jejunum with a crown of Paneth cells showing silver-reducing granules ($\times 100$). *B*, human jejunum showing uninterrupted connections of Paneth cells, with their silver-reducing product filling the lumen of the gland. Note the appearance of the true argentaffin cell in the upper part of the gland on the right.

with their secretory product filling the lumen and giving a reaction of silver reduction similar to that shown by intracellular granules of Paneth cells. The observations in this work are significant for the following reasons: (1) They furnish a reliable method for demonstrating the intracellular and extracellular secretory product of Paneth cells, and (2) they offer additional evidence in support of a concept championed by Klein²² and Bensley²³ to the effect that Paneth cells are zymogenic and have nothing to do with goblet cells. Bizzozero²⁴ claimed to have found transitional forms between Paneth cells and goblet cells. Prenant²⁵ regarded Paneth cells as mucous cells but as specific elements, different from the goblet cells, not as young goblet cells. Hertzog's conclusion is that the Paneth cell gives evidence of being mucoid in character rather than an independent zymogenic cell. One has to agree with Bensley that claims in regard to the specificity of the various methods employed in studies of Paneth cells are unjustifiable and that they have served only to add confusion to this subject. Furthermore, Bensley²³ stated that the difference in appearance of the granules found in Paneth cells is to be given a chemical rather than an architectural interpretation. From studies reported here it is obvious that the tinctorial and reduction effects obtained reflect in reality individual phases in the secretory cycle of the Paneth cell. With the water blue-silver method, depending on the phase of activity of the Paneth cells, granules may not be found at all, may take either water blue or silver or may show only clear vacuolar structures which do not take any stain. With the safranin-silver-water blue method the same results are observed, but the safranin never touches the content of Paneth cells, although it stains the mucous content of goblet cells most selectively. With the potassium bichromate-eosin-silver-water blue method, depending on the phase of activity, granules stain either blue, red or black. The cells of Paneth are secretory zymogenic cells, and they are entirely different from argentaffin cells. Their tinctorial and impregnation features reflect different repeated successive phases of secretion. The ordinary argentaffin cells are not secretory cells, and their tinctorial and impregnation features signify a specific process of rejuvenation of functionally exhausted mucous cells, a process which is not shown by the cells of Paneth.

RESULTS OF STUDY OF THE STOMACH OF THE RABBIT

Each stomach examined was opened immediately and washed with Helly's fluid. Trimmed portions were put in fresh Helly fixative for

22. Klein, S.: *Am. J. Anat.* **5**:315, 1906.

23. Bensley, R. R.: *Anat. Rec.* **2**:92, 1908.

24. Bizzozero, G.: *Arch. f. mikr. Anat.* **40**:325, 1892.

25. Prenant, A.: *Compt. rend. Soc. de biol.* **62**:1125, 1907.

twenty-four hours. Sections were taken from the cardiac portion (including the esophagus), from the fundus and from the pyloric region. The last part was trimmed in such a way that part of the stomach, the pylorus and the duodenal papilla were seen in the same section. The newly devised methods of staining and reduction gave constant, uniform and valuable results.

With the first, or silver-water blue, method, the parietal cells stand out clearly, resembling a bunch of grapes, each with a distinctly outlined (with silver) tubule-like projection directed from each cell toward the lumen and representing apparently the excretory part of the cell (figs. 10 *A* and 11 *D*). The effects of phases of digestion, of different diets and of starvation for twenty-four to seventy-two hours form the comparative material of the present studies. When the first method is applied to the stomach of an animal disposed of six hours after feeding the chief cells appear uniformly agranular and light blue, while the parietal cells vary in shade, some cells being greenish, some rusty yellowish green, some orange brown and some black.

With the third, or potassium bichromate-eosin-silver-water blue, method the differences in appearance of the parietal cells are much more conspicuous. It must be noted here that with hematoxylin-eosin staining the granules in the parietal cells are shown poorly and the individual differences in shades of eosin are too insignificant to be of value. With the third method, parietal cells appear either bluish green, bluish red, violet red, pinkish red or rusty orange brown. It is in cells of the last type that the first appearance of silver-reducing granules is observed (fig. 11 *D*). In the beginning the granules are small and are scattered all over the cell. The nucleus of such a cell loses its fine structural pattern, and its chromatin appears as a confluent mass taking water blue or eosin. In the course of further cytomorphosis the granules become coarser and more numerous, and finally the entire cell becomes loaded with silver-reducing substance to such an extent that no cytoplasmic structure is recognizable and the nucleus is seen only when the level of sectioning passes precisely through its middle. In this stage the argentaffin cell appears as a low conical cell with its broad base in close proximity to the basement membrane. When cut transversally, the cell appears as a triangular cell (fig. 9) placed between zymogenic cells, and the only conclusion that could be drawn from observations on the process of its formation is that it is a parietal cell undergoing specific cytomorphosis. When the parietal cell reaches this stage of cytomorphosis, it begins to rotate and to retract toward the basement membrane (fig. 10 *A*). While retracting, it drags along its outer conical process, which now has the appearance of a tail (fig. 11 *D*). The cell shrinks and flattens and in the final step of retraction takes its position directly at the basement membrane. Its tail still recognizable, the cell is

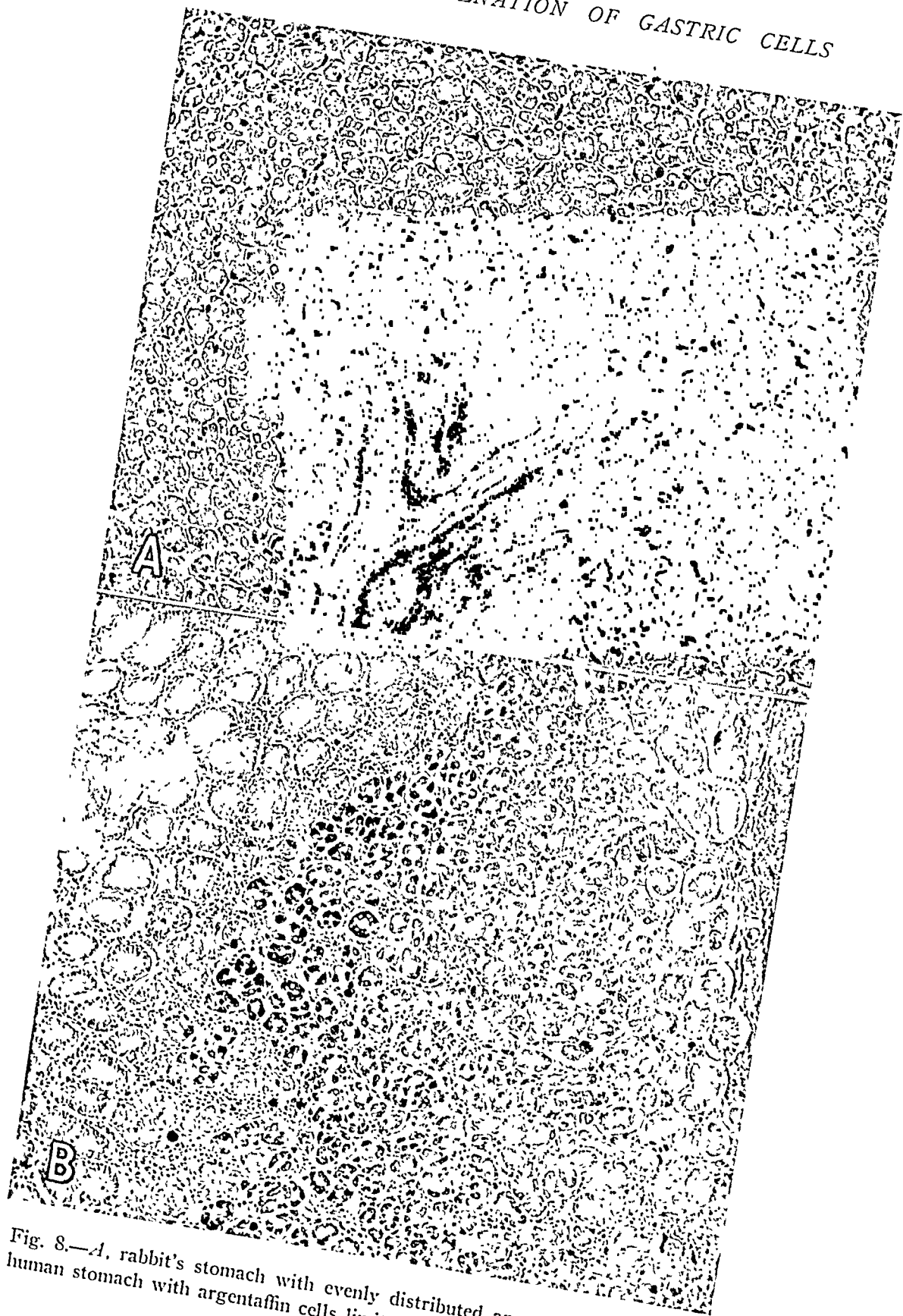


Fig. 8.—*A*, rabbit's stomach with evenly distributed argentaffin cells ($\times 100$).
B, human stomach with argentaffin cells limited to particular areas ($\times 100$).

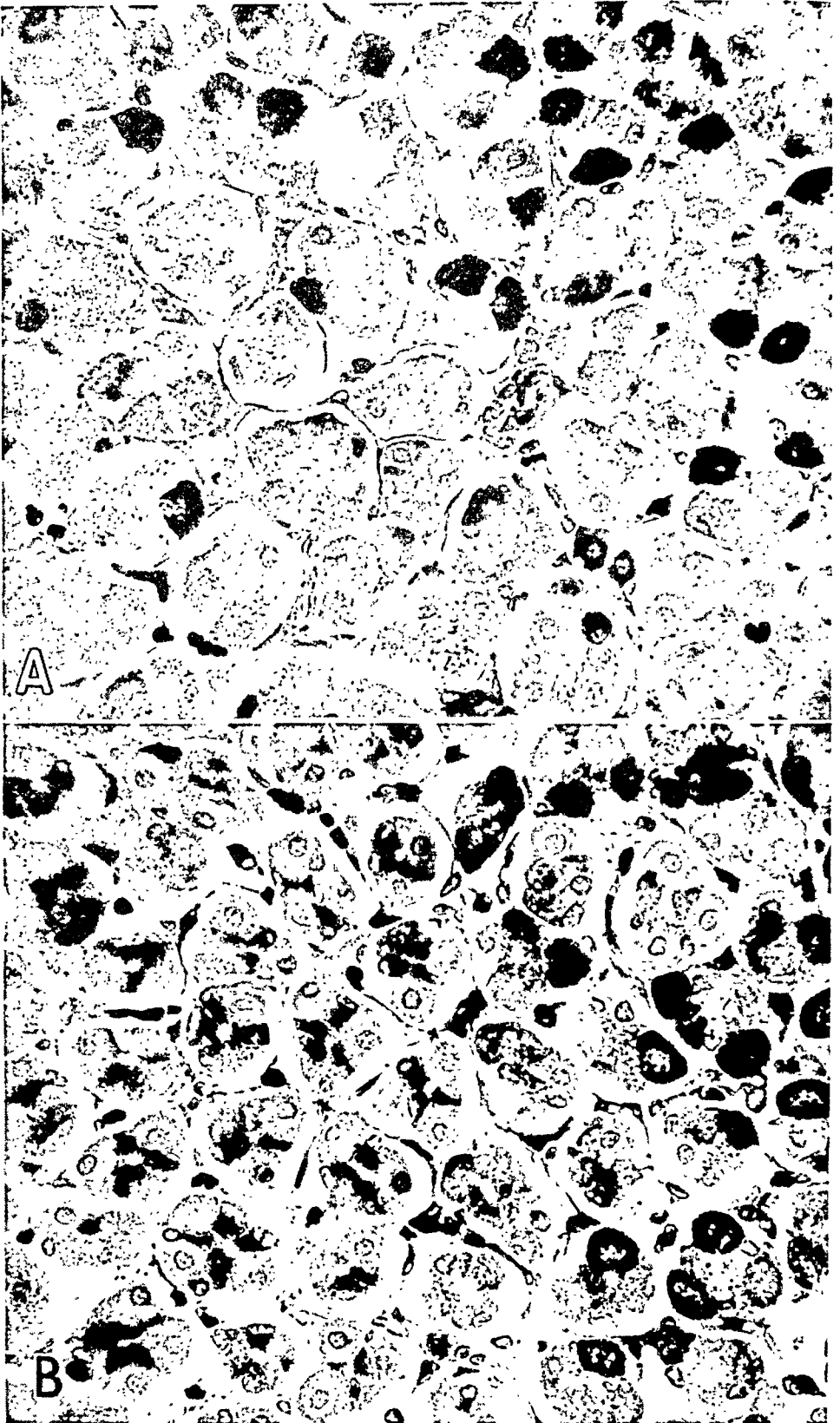


Fig. 9.—*A*, rabbit's stomach six hours after the rabbit was fed, showing early stage of transformation of parietal cells into argentaffin cells. The chief cells are empty ($\times 430$). *B*, rabbit's stomach during starvation, showing an early stage of similar cytomorphosis. The chief cells are filled with granules ($\times 430$).

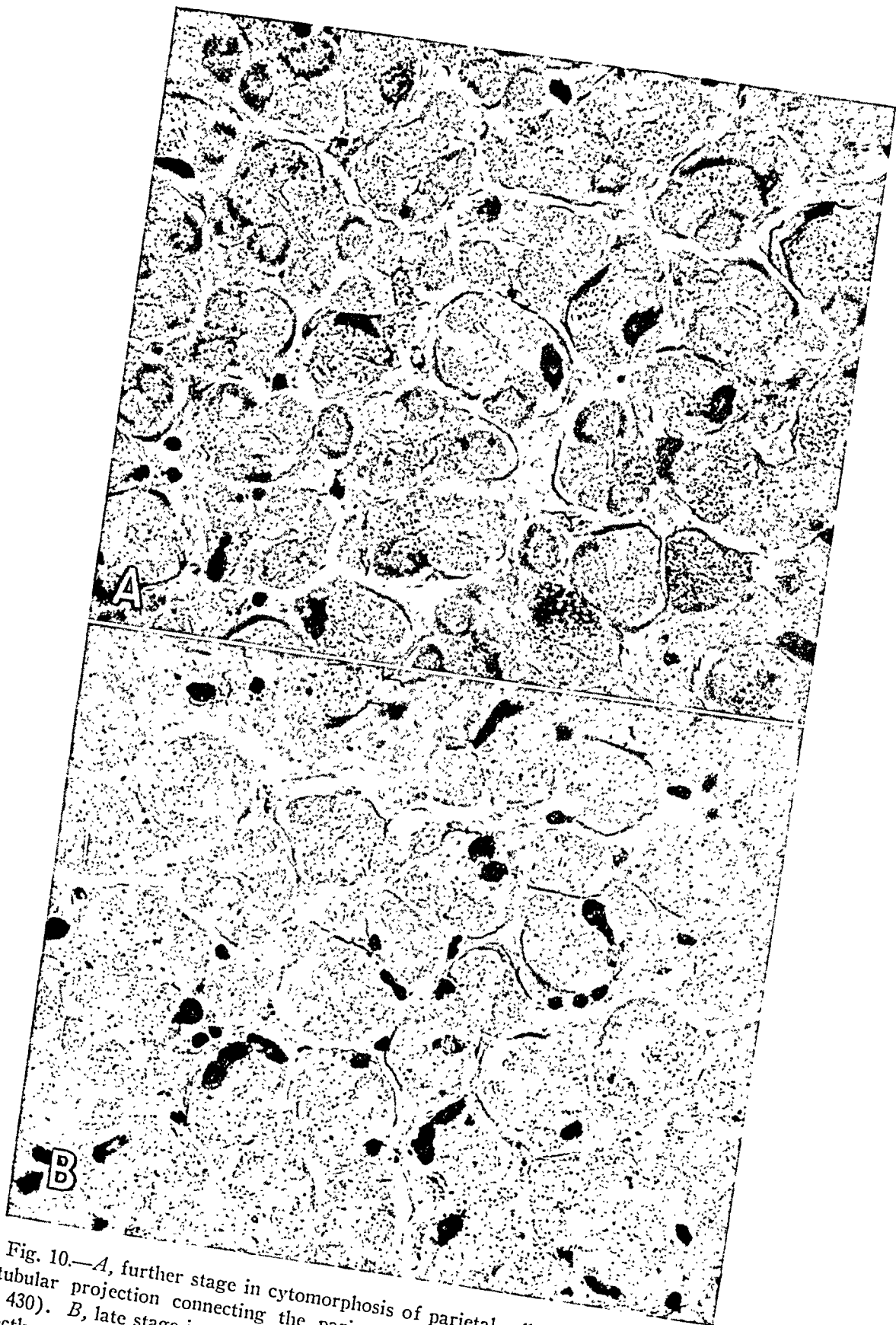


Fig. 10.—*A*, further stage in cytomorphosis of parietal cells. Note in the center a tubular projection connecting the parietal cells with the lumen of the gland ($\times 430$). *B*, late stage in cytomorphosis, with argentaffin cells occupying a position directly at the basement membrane ($\times 430$).

now comma-like in shape (fig. 10 *B*). On further shrinking it is gradually transformed into a small oval or round element (fig. 10 *B*), its argentaffin substance being much more abundant in the subnuclear region. In some of these cells the silver-reducing substance, instead of being jet black, becomes brown and rarefied. As a result of progressive fading, the cell is transformed finally into a chromophobe element which does not take water blue and contains no silver-reducing substance. This chromophobe cell gradually regains the property of staining with water blue, and in the course of further differentiation and reorientation it returns to the normal state of the parietal cell (fig. 12 *I*).

At no time is silver-reducing substance excreted by the argentaffin cell, nor is this substance found in the lumen of the gland or in any other pericellular zone. There are no signs that argentaffin cells have perished, and no evidence is ever found that they are eliminated by necrobiosis or phagocytosis. The histologic appearance of the chief cells and of the parietal cells varies in relation to the phase of digestion. These well known variations have nothing in common with the appearance and disappearance of silver-reducing substance observed in a certain type of parietal cell. The argentaffin substance is not influenced by phases of digestion, by starvation for periods of from twenty-four to seventy-two hours or by types of diet. The chief cells of the stomach never show signs of cytomorphosis similar to that observed in parietal cells. In figure 9 *A* (magnification, $\times 430$ —six hours after feeding) the chief cells are empty while in figure 9 *B* (magnification, $\times 430$ —starvation) they are heavily loaded with zymogenic granules which appear deep blue with a slight touch of silver. These photomicrographs represent two different phases of digestion and show that in spite of the difference in appearance of the chief cells the argentaffin cells are found in both photographs in the same number and are identical in appearance. It proves that neither their number nor their appearance is influenced by the stage of digestion and that the presence of argentaffin substance is not related to the production and secretion of digestive substance. The type, position and distribution of argentaffin cells are not the same everywhere: in one case (fig. 8 *A*— $\times 100$) they are scattered all over and appear as small flattened or oval cells, closely adhering to the basement membrane; in another case (fig. 8 *B*— $\times 100$) they are limited to certain areas and may appear as large triangular cells which differ from the parietal cells only by the presence of argentaffin substance. As in the intestine, mitoses are not found in the gland containing argentaffin cells but are numerous in the gland which shows no argentaffin cells. It is known that an occasional parietal cell of the stomach contains two nuclei, and it is not a coincidence that an occasional argentaffin cell with two nuclei

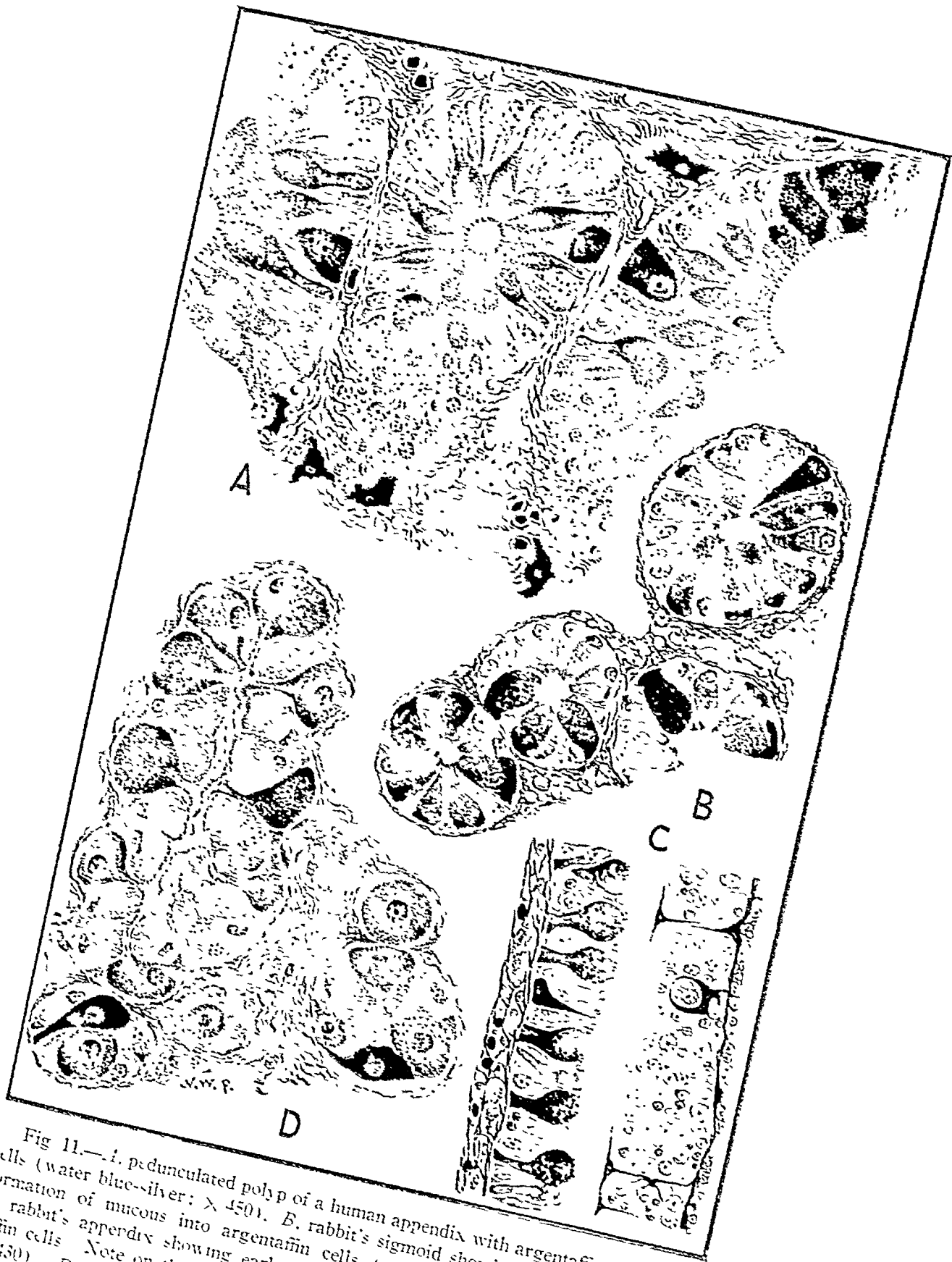


Fig 11.—*A*, pedunculated polyp of a human appendix with argentaffin and Paneth cells (water blue-silver; $\times 450$). *B*, rabbit's sigmoid showing early stage of transformation of mucous into argentaffin cells (safranin-water blue-silver; $\times 430$). *C*, rabbit's appendix showing early stage of transformation of goblet into argentaffin cells. Note on the right side red-black histiocytes (eosin-water blue-silver; $\times 430$). *D*, rabbit's stomach showing transformation of parietal into argentaffin cells (water blue-silver; $\times 450$).

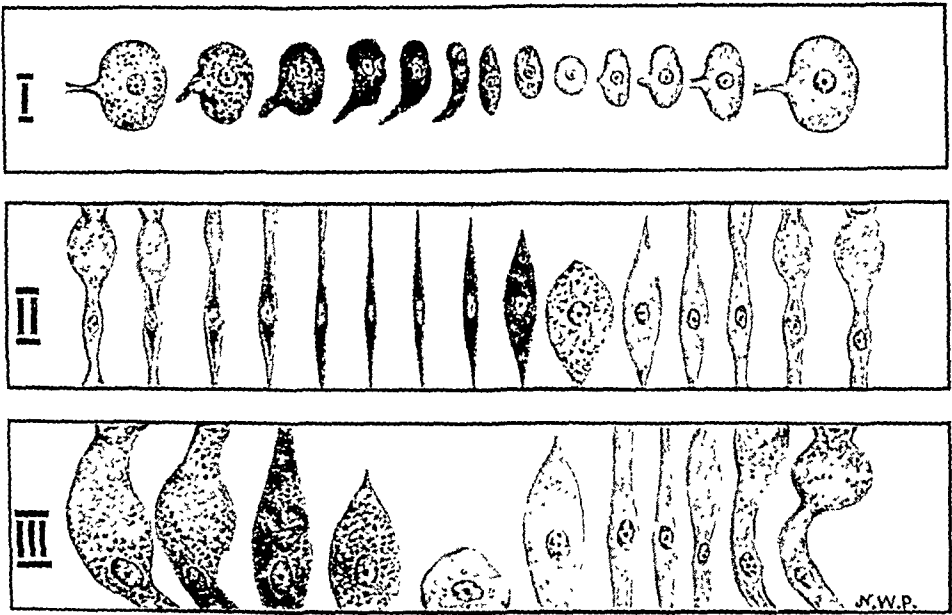


Fig. 12.—Rejuvenation cytomorphosis of (I) parietal cells of the stomach, (II) goblet cells of the small intestine and (III) goblet cells of the sigmoid.

is also found and only in the stomach. This is certainly a substantial argument in support of a genetic relationship between the parietal and the argentaffin cell of the stomach. Summarizing, then, the essential results of this work, one may conclude that the cytomorphosis observed in certain types of parietal cells is identical with that shown by the mucous cells of the intestine. It signifies rejuvenation of the functionally exhausted parietal cells and serves to explain a number of unsolved problems connected with the activity of parietal cells in normal and in pathologic conditions.

The space allotted does not permit discussion of other details, and only the following brief notes will be added. Brunner's glands take safranin and appear pinkish rose. Their secretory ducts contain both goblet and argentaffin cells, although neither of these types of cells is found in the gland itself. Pyloric and cardiac mucus-secreting glands show cytomorphosis of the rejuvenation type, this being especially pronounced in the stomach of the rat.

SUMMARY AND CONCLUSIONS

This work represents one more effort to shed light on hitherto unsolved problems concerning the origin and function of the argento-chrome cells of the gastrointestinal tract, which were discovered in 1870 by Heidenhain²⁶ and later studied by Grutzner and Menzel,²⁷ Nussbaum,²⁸ Stohr²⁹ and others. In this work I have followed the traditions of my teacher, the late N. K. Kultschitzky,³⁰ who was an outstanding pioneer in this field of research. In approaching the problem careful attention was given to the work of all previous investigators. It is regrettable that discussion of their contributions is prevented by lack of space. It is satisfying, however, to know that there are in the literature reviews by Macklin and Macklin,¹ Schaffer,³¹ Babkin,³² Alvarez³³ and others of the histophysiology and pathology of the alimentary tract which are unsurpassed in their impartiality and completeness and which make unnecessary repetition here.

26. Heidenhain, R.: *Arch. f. mikr. Anat.* **6**:368, 1870.

27. Grutzner, M., and Menzel, H.: *Arch. f. d. ges. Physiol.* **20**:395, 1879.

28. Nussbaum, M.: *Arch. f. mikr. Anat.* **16**:532, 1879.

29. Stohr, P.: *Arch. f. mikr. Anat.* **20**:221, 1882.

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31. Schaffer, J.: *Das Epithelgewebe*, in von Möllendorff, W.: *Handbuch der mikroskopischen Anatomie des Menschen*, Berlin, Julius Springer, 1927, vol. 2.

32. Babkin, B. P.: *Die äussere Sekretion der Verdauungsdrüsen*, in Gilde-meister, M., and others: *Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere*, ed. 2, Berlin, Julius Springer, 1928, vol. 15.

33. Alvarez, W. C.: *The Mechanics of the Digestive Tract*, ed. 2, New York, Paul B. Hoeber, Inc., 1928.

From the studies presented in this paper it appears that argento-chrome cells are not exocrine, endocrine or neurocrine. The objective findings in studies of 92 rabbits under normal and experimental conditions and of a considerable variety of human tissues are summarized in the following conclusions:

1. New, simple, rapid methods of staining and reduction have been developed which have proved of particular value in demonstrating a genetic relationship between the mucous and argento-chrome cells of the intestine and between the parietal and argento-chrome cells of the stomach. The methods formerly employed in the studies of argentaffin cells are cumbersome and time consuming and are worthless in demonstrating this genetic relationship.

2. Under normal circumstances the mucous cell of the intestine passes through successive phases of secretory activity repeatedly, and when finally the cell reaches the stage of functional exhaustion it does not perish. It becomes refractory, loses its response to normal and to artificially applied secretory stimuli and undergoes a rearrangement or cytomorphosis, manifested by the appearance, accumulation and gradual disappearance of substances which have the property of reducing metallic salts. In the course of this cytomorphosis the cell changes its position, dedifferentiates and returns eventually to the state of a normal secreting cell. This cytomorphosis of the functionally exhausted mucous cell is designated as a phenomenon of functional rejuvenation, and the entodermal argento-chrome cell may be called a rejuvenocyte.

3. The rejuvenation is accompanied by certain cytoplasmic and nuclear changes, and the cytomorphosis observed in the course of rejuvenation is a manifestation of specific intracellular chemical processes and bears no relation to the elaboration or secretion of any product in the sense of exocrine or endocrine secretion.

4. The existence of such cytomorphosis is supported by the following findings:

- (a) With the methods employed, definite intermediary forms are found between the functionally exhausted mucous cells, argentaffin cells and chromophobe cells.

- (b) The silver-reducing substance is never excreted into the lumen of the intestine and is not found extracellularly.

- (c) These cells cannot be forced to evacuate this substance by application to the mucosa of drastic irritants and other chemicals, and pilocarpine, applied locally or intravenously, fails to affect these cells.

- (d) The number of argentaffin cells is not influenced by phases of digestion, by types of diet or by nineteen different chemical substances that were applied.

(*e*) The number of mitoses is in inverse relation to the number of argentaffin cells found in the same area, indicating that whenever rejuvenation fails regeneration by mitosis is called on to compensate this failure by the production of new cells destined to reach the same goal of functional efficiency.

(*f*) The epithelial argentaffin cells are never found migrating through the basement membrane, and extraglandular argentaffin cells are either mesenchymal or ectodermal.

(*g*) When found extraglandularly in the appendix or ileum, some argentaffin cells may be of epithelial nature, for the lymphoid tissue of the appendix and of Peyer's patches is in reality lymphoepithelial tissue, which resembles closely the lymphoepithelial tissue of the bursa of Fabricius. This offers a new approach to studies on the origin of extraglandular carcinoid tumors.

5. Argentaffin cells are found in benign pedunculated polyps and in some portions of malignant tumors of the intestine. In their origin and significance they are similar to argentaffin cells in the normal intestine, and it may be said that whenever there are in tumors mucous cells capable of function and rejuvenation there are found argentaffin cells also. The characteristic feature of benign polyps is that the rejuvenation cycle proceeds normally and mitoses are rare. When, owing to any cause, rejuvenation fails, the cells begin to regenerate, and the process of exuberant regeneration may take the course of a malignant neoplasm. It is not the degree of glandular differentiation that retards the malignancy of a growth. It is the ability of the cells to function and to rejuvenate that makes a tumor orderly and slow growing.

6. When mucous cells are called on to perform their function and to rejuvenate under unfavorable circumstances, such as advanced age, continuous irritation and stasis—for instance, in the colonic segment above a carcinomatous obstruction—the cycle of rejuvenation of the functionally exhausted cells is interfered with or interrupted. As a result of this, the endogenous product of their gradual disintegration is taken up by macrophages, which are seen at first only around the affected gland but later on in the corresponding deep part of the submucosa. This leads to patchy pigmentosis or melanosis, which is not found below the obstruction, where rejuvenation is not interfered with and consequently proceeds in the usual way. This serves to explain the origin and topographic peculiarities in the distribution of intestinal melanosis. The mucosa of the colon and appendix consists chiefly of mucous cells, while the mucosa of the small intestine consists of different epithelial cells and shows a much smaller number of mucous cells scattered here and there. This makes understandable why melanosis above the ileocecal valve is hardly ever observed.

7. The methods employed show the hitherto undemonstrable capacity of Paneth cells to reduce metallic salts. The results obtained indicate that in certain phases of secretory activity the granules of Paneth cells are impregnable with silver nitrate and that the same silver-reducing product of secretion is demonstrable with utmost clarity in the lumen of the gland. The cells of Paneth are secretory zymogenic cells, and they do not show the cycle of rejuvenation observed in mucous cells. They have nothing in common with mucous and argentaffin cells.

8. The methods employed demonstrate a genetic interrelationship between parietal cells of a certain type, argentaffin cells and chromophobe cells of the stomach. They show that the functionally exhausted parietal cell, instead of perishing, undergoes cytomorphosis, and the argentaffinity observed signifies the phenomenon of functional rejuvenation. As in the intestine, the argentaffin substance is not excreted by the cell and is never found outside the cell. The number of argentaffin cells is not influenced by phases of digestion, and argentaffinity is not related to the production and excretion of exocrine or endocrine substance. When passing through the stage of rejuvenation the parietal cell is relieved of its specific functional duty. With ordinary methods, the parietal cell in the early stages of rejuvenation does not reveal peculiar features; in other words, these older methods are unable to reflect the state and behavior of parietal cells in various physiologic and pathologic conditions.

9. Both pyloric and cardiac mucus-secreting glands show argentaffin cells of the rejuvenation type.

10. Excretory ducts of Brunner's glands are furnished with the goblet cells, and they show the presence of argentaffin cells.

11. In control studies the new methods were applied to all tissues of the animal body, and the results obtained are very interesting. Method 3 is especially valuable when applied to the hypophysis, in which it shows four distinct type of cells: chromophobe, light blue, red and argentaffin. The significance of such tinctorial and reduction effects is a problem in itself. These methods also permit differentiation of mesenchymal elements in relation to the phase of their life and activity, and they are valuable for studies of the intraepidermal cells of Langerhans which with these methods behave like regular histiocytes.

In conclusion: There are cells of merocrine secretion (the mucous cells of the gastrointestinal tract and the parietal cells of the stomach) which have the biologic faculty of maintaining their longevity and usefulness by cyclic returns, when functionally exhausted, to their primitive afunctional state, each return being followed by progressive differentiation and full recovery of their original efficiency. Argentaffinity, or metallaffinity, demonstrable with histochemical methods, reflects simply a

stage in the rejuvenation of certain functionally exhausted and refractory entodermal cells and bears no relation to exocrine, endocrine or neurocrine secretion. The significance of the observations reported with the new methods is discussed with reference to the following problems: (1) the general life cycle of certain highly differentiated cells; (2) the functional endurance of such cells in the absence of any signs of regeneration; (3) inability of some fully developed cells which have approached the rejuvenation stage to react to normal and artificially applied stimuli; (4) normal compensatory regeneration and focal neoplastic proliferation caused by any intrinsic or extrinsic factor interfering with the rejuvenation cycle or causing a cessation of it; (5) melanosis coli, precipitated by local disturbances, with rejuvenation of the goblet cells; (6) the lymphoepithelial nature of the lymphoid tissue of the appendix and of Peyer's patches and (7) additional evidence in favor of the zymogenic nature of the cells of Paneth.

EFFECT OF THYROID FEEDING ON THE REMOVAL OF CHOLESTEROL

LEO ZON, M.D.

BALTIMORE

It is known from the work of Murata and Kataoka,¹ Liebig,² Turner,³ Page and Bernhard⁴ and Menne, Beeman and Labby⁵ that administration of desiccated thyroid and thyroid-stimulating iodine compounds may prevent experimental atherosclerosis in rabbits. It is possible that, in addition to the lowering of the level of the blood cholesterol described by many authors, there is a direct stimulation of the macrophages in the aortic wall which, as has been shown by Anitschkow⁶ and Leary,⁷ take up deposited cholesterol and tend to dispose of it. In either case, whether the mechanism of thyroid protection from atheroma is a lowering of the level of blood cholesterol or some local effect, interest attaches to the effect of thyroid on the ability of cells to dispose of or destroy cholesterol; for any reduction in blood cholesterol must be caused by some cellular process in the liver or in the reticuloendothelial system. That the reticuloendothelial and macrophage system is intimately concerned in the handling of cholesterol in the body is indicated by the work of Anitschkow, Chalataw,⁸ Kimmelstiel and Laas,⁹ Thannhauser and Magendanz¹⁰ and others.

In order to reveal the action of thyroid on cells phagocytosing cholesterol, a study was made of the effect of thyroid feeding on the removal of experimental intracutaneous cholesterol deposits.

METHODS AND TECHNIC

The cholesterol was injected in two different ways, suspended in water and suspended in olive oil. The water suspension was made by dissolving 0.5 Gm. of cholesterol crystals in 10 cc. of acetone. This was added to 50 cc. of a 0.5 solution of gelatin in water. The acetone was removed and the suspension concen-

From the United States Marine Hospital.

1. Murata, M., and Kataoka, S.: *Verhandl. d. jap. path. Gesellsch.* **7**:27, 1927.
2. Liebig, H.: *Arch. f. exper. Path. u. Pharmakol.* **195**:265, 1930.
3. Turner, K.: *J. Exper. Med.* **58**:115, 1933; **62**:721, 1935.
4. Page, I., and Bernhard, W. G.: *Arch. Path.* **19**:530, 1935.
5. Menne, A.; Beeman, J., and Labby, D.: *Arch. Path.* **24**:612, 1937.
6. Anitschkow, N.: *Verhandl. d. deutsch. path. Gesellsch.* **23**:473, 1928.
7. Leary, T.: *Arch. Path.* **21**:419 and 459, 1936.
8. Chalataw, S.: *Beitr. z. path. Anat. u. z. allg. Path.* **47**:85, 1914.
9. Kimmelstiel, P., and Laas, E.: *Beitr. z. path. Anat. u. z. allg. Path.* **93**:147, 1934.
10. Thannhauser, S., and Magendanz, A.: *Ann. Int. Med.* **11**:1662, 1938.

trated by boiling. After the suspension had been filtered through dense paper the concentration of cholesterol was 12 mg. per cubic centimeter. Such a suspension may be injected through a 26 gage needle. The oil solution contained 0.5 Gm. of cholesterol in 12 cc. of olive oil. This was heated till clear and then was chilled and shaken until very fine crystals formed. It was injected at room temperature.

In preliminary experiments it was found that more than 0.6 cc. of the water suspension and more than 0.2 cc. of the oil suspension when injected intradermally would produce a nodule the contents of which would slough out in seven to eight days. Sloughing out of the nodule occurred also if the injection was given too superficially. If the cholesterol was injected beneath the dermis, it was spread by the movement of the skin to such an extent that no definite nodule formed.

Four adult rabbits were given intradermal injections of 0.2 cc. and 0.4 cc. of the water suspension in the clipped flank. There was a control injection of 0.5 cc. of the gelatin. The cholesterol produced firm lentil-shaped nodules, 0.5 and 0.6 cm. in diameter, while the control injection vanished within twenty-four hours. Tablets of commercial iodothyroglobulin were ground into a suspension and administered by means of a soft rubber catheter daily to 2 of the 4 rabbits. The rabbits received iodothyroglobulin equivalent to 3 to 5 grains (0.2 to 0.3 Gm.) of desiccated thyroid daily. This dosage produced loss of weight and emaciation. Such doses have been shown by Turner³ and by Menne, Beeman and Labby⁵ to be sufficient to protect against atheroma.

The second group was composed of 10 healthy rabbits which had been discarded after previous bleedings and experiments. Each of these animals was given five injections intradermally, making a row parallel to the spine. Fifteen days later, another series of five lesions was placed on the opposite side in every animal in this group except 3. Each injection consisted of 0.15 cc. of the olive oil suspension. Control injections of 0.15 cc. of olive oil were given. Test rabbits received 4 to 6 grains (0.25 to 0.4 Gm.) of desiccated thyroid daily. When the drop in weight became too rapid, the administration of thyroid was discontinued for several days. There were 5 test rabbits and 5 controls. Two test animals died on the eleventh day from overdosage of thyroid. Lesions were removed after they had been present for fifteen days and after thirty days. Lesions from 1 test animal and 2 controls were removed at twenty-eight days and fifty-eight days, respectively.

RESULTS

The 4 rabbits given the watery suspension showed but little inflammatory change about the points of injection. The 0.2 cc. nodules were removed from all 4 rabbits after fifteen days. At this time the nodules had decreased from about 5 mm. to about 3 mm. in diameter. There was no difference in the size of nodules on controls and thyroid-fed animals. One test animal died two days later, and the 0.4 cc. lesion was removed. The remaining 3 rabbits were followed for two weeks more, until the lesions were barely palpable. The lesions on the 3 animals were almost identical in size at this time, again showing no effect of the thyroid feeding.

After injection of the oil suspension there occurred an inflammatory area 1 cm. in diameter, which in the course of several days contracted to a small nodule, 5 to 6 mm. in diameter. The impression was gained that in the thyroid-fed rabbits the olive oil control and the first inflam-

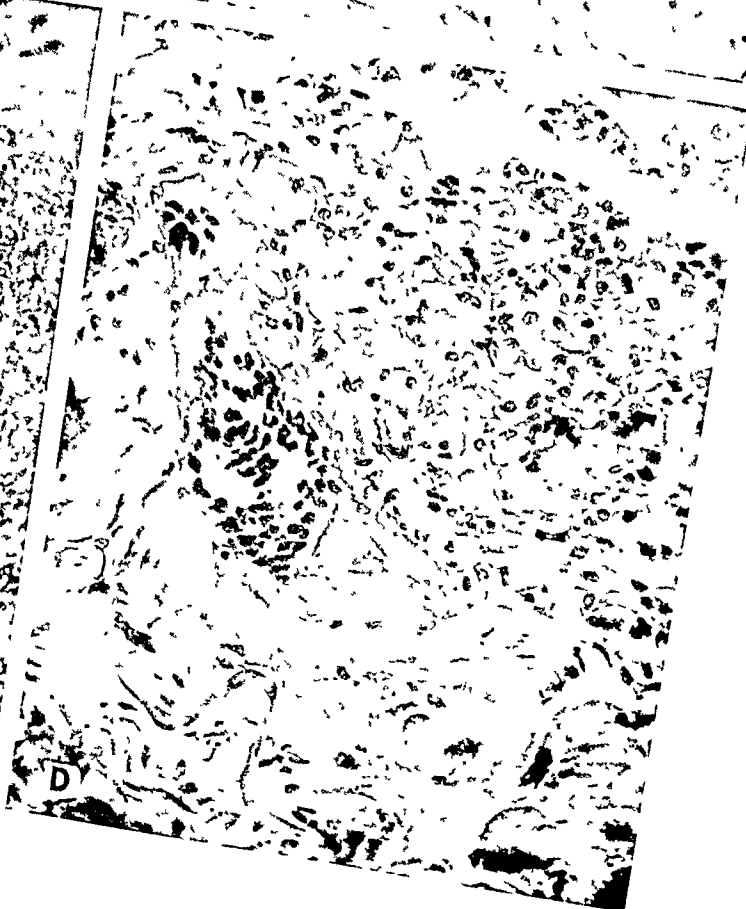
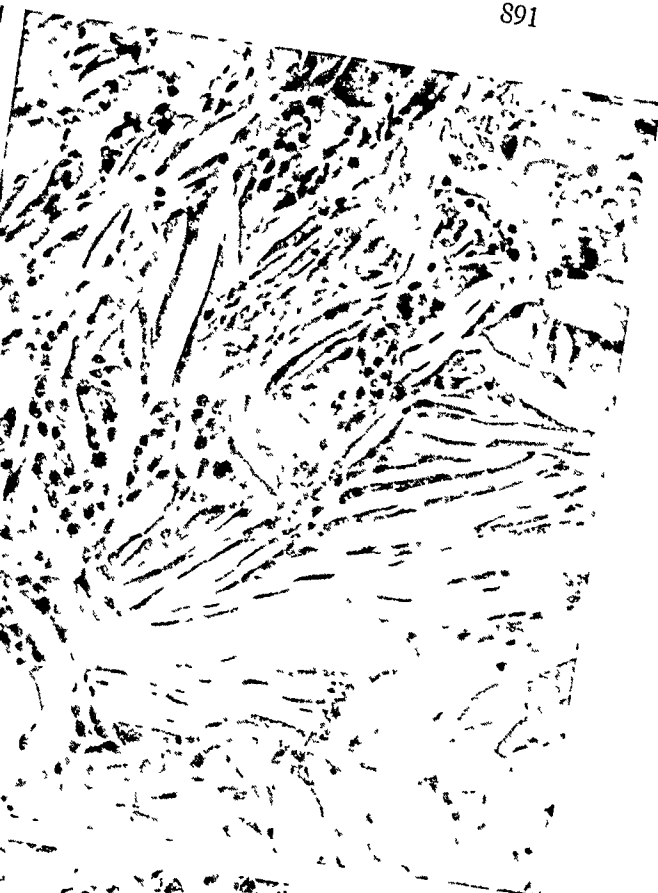
EXPLANATION OF FIGURE

A, lesion eleven days old from a test rabbit (oil suspension); hematoxylin and eosin; $\times 175$. The photomicrograph shows the cholesterol spaces with protein between them but with no cellular reaction. The darker areas are collagen fibers, which take a deep hematoxylin stain.

B, lesion two weeks old from a control rabbit (oil suspension); hematoxylin and eosin; $\times 540$. The histiocytes are shown collecting about and between the cholesterol crystals and forming giant cells.

C, lesion fifteen days old from a test rabbit (water suspension); frozen section stained with sudan III and photographed between partially crossed polarizers; $\times 120$. It shows the distribution of sudan-stained macrophages about the central crystal deposit. It may be noted that although the macrophages stain deeply with sudan III there is little anisotropic material within them.

D, lesion thirty days old from a test rabbit (water suspension); hematoxylin and eosin; $\times 540$. The very large giant cell with eosinophilic peripheral cytoplasm and the finely granular cytoplasmic zone is shown together with lipoid-laden macrophages and smaller giant cells.



matory phase of the lesions disappeared more rapidly than in the controls. After about a week the lesions on control and test animals became the same size and remained so. There was some variation in size between lesions of the same duration on the same animal. One control rabbit of this group had unusually large lesions, which remained larger than those of the other controls.

With the exception noted, it was clear that there was no significant difference in the size and character of the lesions on control and test animals.

MICROSCOPIC OBSERVATIONS

The microscopic observations are based on sections from: (a) fifteen day lesions of 2 control and 2 test animals; (b) eleven day lesions from 2 test animals; (c) fifteen day lesions from 2 test animals and 3 controls; (d) thirty day lesions from 2 test animals and 3 controls; (e) twenty-eight and fifty-eight day lesions from 1 test animal and 2 controls. Each lesion was studied in both frozen and paraffin sections. The frozen sections were examined with sudan IV and between polarizers.

During the first week the lesions showed little reaction about the deposits of crystals. In several lesions there were numerous polymorphonuclear leukocytes, but their presence was interpreted as a reaction to accidental infection. *A* in the figure shows the absence of cellular reaction after eleven days. There is only a slight suggestion of the marked necrosis described in such lesions by Basten.¹¹

After the first week there was mobilization of histiocytes. These cells, which are shown in *B* in the figure, applied themselves to the surfaces of the cholesterol, sent out processes which enveloped the crystals and in other places fused to form giant cells. In the lesions produced by the water suspension many of these early cells degenerated, forming a cellular débris, which was removed by later cells. In the lesions produced by the oil suspension (*B* in the figure) this degeneration was absent. Aside from this difference the oil suspension lesions ran parallel with the water suspension lesions. The cytoplasm of the histiocytes and of the giant cells they formed was at first eosinophilic with a very homogeneous appearance. In the lesions from two to three weeks old, "foamy" areas with neutral staining properties appeared within individual cells and within the giant cells. The giant cell in *D* in the figure shows this foamy change in the central region occupied by the nuclei. In almost every case the nuclei of the giant cells were within this area and usually distributed along its periphery. This tendency may be clearly seen in *D*. The foamy areas of the giant cells under the highest resolving powers of the microscope were identical with the contents of the so-called "foam cells." In the oldest lesions

11. Basten, G.: Virchows Arch. f. path. Anat. **220**:776, 1915.

there were few giant cells, the predominating cell being a rounded, well filled macrophage with foamy or granular, neutral cytoplasm.

Frozen sections of the lesions with an early macrophage response showed numerous intracellular sudanophilic droplets, which were for the most part optically inactive. This is shown in C in the figure. This lesion was taken from a rabbit which received the watery suspension of cholesterol. There is, therefore, no possibility that these droplets were phagocytosed olive oil. It must follow, then, that one of the earliest steps in the removal of cholesterol is the mobilization of lipoids into macrophages for the purpose of either esterification or emulsification. It has been pointed out by Kutschera-Aichbergen¹² and Lison¹³ that decision as to the chemical nature of the lipoids from their staining properties is hazardous. From their indifference to polarized light one might believe that the fine sudan-staining droplets are for the most part fats and fatty acids.

The older lesions showed many intracellular optically active droplets, many of which exhibited the polarization cross. The presence of the latter droplets has led to the idea expressed by Basten¹¹ that esterification precedes phagocytosis. In these sections there were also many extracellular droplets showing the polarization cross, which possibly indicated extracellular esterification.

It was hoped that careful microscopic study would reveal some difference between the lesions of the thyroid test animals and those of the controls. This difference might be expected in the size and number of histiocytes and their degree of development into "foam cells." There also might be differences in the size, number and character of the giant cells. Finally the amount of optically active material left in the form of crystals could be readily compared in frozen sections in control and test animals. Careful comparison, however, revealed that none of these differences were present.

COMMENT

It may be objected that removal of pure cholesterol is not analogous to the removal of lipoids from the aorta, for this contains a large proportion of esters. It is possible that the removal of cholesterol has as one of its slower steps, esterification, which may be unaffected by the general metabolic activity of the phagocytic cells. Thannhauser and Magendanz¹⁰ stated that xanthomas become relatively higher in free cholesterol with age, which indicates that esters may be removed more readily than free cholesterol. In the lesions examined, however, it is estimated that from one third to one half of the original crystalline material was changed into droplet form and taken up into phagocytic cells. If one may believe that cholesterol is well on its way to its

12. Kutschera-Aichbergen, H.: *Virchows Arch. f. path. Anat.* **256**:569, 1925.

13. Lison, L.: *Bull. d'histol. appliq. à la physiol.* **10**:237, 1933.

ultimate local fate by the time it is taken up into granular phagocytes, it is clear that thyroxin has had ample opportunity to exhibit its effects on these later stages of the removal process. The numerous extracellular droplets showing the polarization cross were present in almost the same numbers in the test and control lesions.

Thannhauser and Magendanz¹⁰ pointed out that it is likely that cholesterol absorbed or synthesized in metabolic processes is excreted unchanged. This indicates that there is probably little cellular destruction of the cholesterol molecule. Such a conception fits well with the finding that thyroxin does not stimulate the removal process, for the effect of thyroxin is on cellular oxidation processes. The numerous very fine sudanophilic optically active droplets seen in frozen sections and the fine foam structure of the phagocytic cells in paraffin sections suggest that the essential process of removal is a purely physical one of emulsification and possibly solution in fine fat droplets.

While this material demonstrates clearly the conversion of cholesterol from crystalline form into intracellular droplet form, it does not throw much light on the ultimate removal of the cholesterol. Since the appearance of the crystals is so different from that of the optically active droplets and since the dispersion of the cholesterol influences its optical activity, it is impossible to estimate how much of the original deposit of cholesterol has been removed from the lesion.

Beidermann and Hoefer¹⁴ showed that lipoid-laden macrophages retain the power of migration. Many foam cells were found some distance from the main deposit of cholesterol, but this was interpreted as being due to a dissemination of the injected material rather than to migration of macrophages. The only clue to the mechanism of removal suggested by this material is the finding that some of the older lesions showed large granular macrophages which appeared to have disintegrated and thus discharged their highly emulsified contents into the lymph spaces.

The observations described indicate that there is no marked and immediate effect exerted by thyroid on the removal or destruction of cholesterol. There may, of course, be some less marked, slower effect which can be detected only by quantitative measurement. In view of the absence of any thyroid stimulation of the removal of cholesterol by macrophages it seems that it would be more logical to seek the mechanism of this protection from atheroma in an effect on excretion or synthesis.

CONCLUSION

Thyroid feeding does not accelerate the removal of cholesterol from intracutaneous experimental deposits in the rabbit.

14. Biedermann, W., and Hoefer, K.: *Arch. f. exper. Zellforsch.* **10**:93, 1930.

Case Reports

PRIMARY FIBROSARCOMA OF THE BRAIN

LILLIAN COTTRELL, M.D., MINNEAPOLIS

To the present time only 6 tumors composed of fibroblastic tissue have been reported as primary in the brain substance. Histologically they resembled fibroblastoma occurring elsewhere in the body, although their origin has been a moot question. It seems permissible to report another such tumor, not only because of the rarity of tumors of this type but also because of the additional evidence available as to their probable origin.

The reports of the first 4 primary fibroblastic tumors of the brain to be recorded in the literature have been reviewed by Baker and Adams¹ and will be summarized only briefly in this report. Bailey² reported 2 tumors of this type. One of these was studied by Mallory. The first tumor occurred in the right temporal lobe of a 42 year old woman as a semiopaque cartilaginous mass adherent to the dura. Histologically it was typical of fibrosarcoma, being composed of spindle-shaped cells separated by reticulin and collagen. Bailey's second tumor was an operative specimen removed from the right lateral ventricle of a 19 year old youth. It consisted of streams of spindle-shaped cells which had a delicate cytoplasm and oval or elongated nuclei containing dustlike chromatin material. Collagen was abundant in the degenerative areas.

Mallory³ described a primary fibroblastic tumor in the right frontal lobe of a 33 year old man. It measured 5 cm. in diameter and was of an unusual firmness and whiteness. Histologically it was a slow-growing fibrosarcoma.

Alpers, Yaskin and Grant⁴ added a fibroblastoma removed from the right frontotemporal region of a 52 year old man. It was an encapsulated white fibrous tumor, composed of loosely packed cells and myxomatous tissue. The rich intercellular substance consisted of fibrous tissue and fibroglia. Areas of degeneration and blood vessels were numerous. The neoplastic cells in close association with the walls of the blood vessels were in a stage of proliferation and were assumed to be "centers of growth" for the tumor.

Baker and Adams¹ reported a primary fibroblastoma found at autopsy in the right frontal lobe of a 10 year old girl. The tumor measured 5 cm. in all diameters and was extremely firm and sharply circumscribed but not encapsulated. On the cut surface it was white, semiopaque and almost gritty. It showed no attachment to the dura. The tumor was uniformly composed of fine and coarse strands of intertwining collagenous fibers that extended in all directions and stained readily with

From the Division of Nervous and Mental Diseases, University of Minnesota.

1. Baker, A. B., and Adams, J. T.: *Am. J. Path.* **13**:129, 1937.

2. Bailey, P.: *Arch. Surg.* **18**:1359, 1929.

3. Mallory, T. B.: *New England J. Med.* **203**:177, 1930.

4. Alpers, B. J.; Yaskin, J. C., and Grant, F. C.: *Arch. Neurol. & Psychiat.* **27**:270, 1932.

azocarmine. In some areas these intercellular fibers were fused to form homogeneous bundles, which had a hyaline appearance. In certain parts of these bands occurred plaque-like thickenings. Interspersed among the fibers of collagen were a moderate number of irregular cells. The majority were elongated and contained scanty cytoplasm with a finely granular nucleus. The brain tissue adjacent to the neoplasm showed extensive glial and microglial reaction, but within the tumor only microglia cells were present. Blood vessels were numerous in all sections but especially in the more cellular areas. These vessels varied from the more frequent endothelium-lined cavities to the less frequent vessels having walls typical of cerebral arteries. In no case, however, were there any proliferative areas comparable to the "centers of growth" described by Alpers, Yaskin and Grant.⁴ Numerous hemorrhages were present, most of which were not perivascular, although there were a few so-called ring hemorrhages.

Meyer and Scheller⁵ reported a fibromyxoma which protruded from the left temporal region of a 25 year old woman. This fluctuant mass had been obvious since a few months after birth, with no clinical symptoms until the age of 18. At autopsy the tumor was cystic and very large, replacing the greater part of the right parietal area. It extended from the frontal region to the transoccipital sulcus. The right side of the cerebrum and the basal structures were markedly distorted, and the convolutions were flattened. The tumor was not attached to the meninges. Externally the new growth appeared grayish and glassy, and was of a tough elastic consistency. Its cut surface contained numerous cysts of all sizes. The solid portions of the neoplasm were variable in appearance, some being whitish and calcified, while others were hemorrhagic, mucinous and soft. Microscopically, the tumor was composed of connective tissue septums interspersed with young fibroblasts. To the authors, the characteristic feature of this new growth was the presence of degenerative fibroblasts, which had become vacuolated and filled with a mucoïd substance. The authors expressed the belief that these vacuoles ruptured, discharging their mucin, and were therefore the source of the mucin found in the interstices. The tumor was vascular, and from the adventitia of the vessels many fine collagenous strands extended outward into the adjacent tissue. This outgrowth of connective tissue from the adventitia of the blood vessels resembled the "centers of growth" described by Alpers, Grant and Yaskin.⁴ The tumor both invaded and compressed the surrounding tissue. Other features observed were lipomas of the meninges and small cysts of the kidney.

REPORT OF A CASE

A white man aged 52 years had been somewhat uncooperative and careless for the past five years. One year prior to his admission to the hospital he began to complain of headache and periods of dizziness. He continued to work, however, until five months prior to his admission, at which time he suddenly suffered a convulsive seizure, associated with unconsciousness lasting for forty-five minutes. The patient had complete amnesia for this attack. Following this spell he remained at home for seven weeks, during which time he complained of weakness and malaise. The headache, which had left following the seizure, returned. His behavior throughout this time remained normal. The patient then returned to work, but it was noticed that his judgment was becoming poor. Two months prior to admission he became peculiarly quiet and began to stumble in his speech.

5. Meyer, H. H., and Scheller, H.: *Virchows Arch. f. path. Anat.* **300**:473, 1937.

He would express himself with the wrong words, although he seemed to know what he wanted to say. This defect in speech was not continuous but came and went, many days passing in which it was not present at all.

When first seen by a physician, he complained of a heavy feeling in his head. The results of an examination of the cranial nerves were negative. There was weakness of the right upper extremity and absence of the patellar reflex on the same side. The Chaddock, Oppenheim and Gordon signs were observed on the right. Incoordination was noticed in the performance of the right finger to nose test. Superficial and deep sensation were intact. The rest of the neurologic examination gave negative results. The patient presented mild motor aphasia, an apathetic emotional attitude and some diminution in attention. Laboratory studies gave negative results. Roentgen studies after injection of air into the lateral ventricles revealed them to be displaced to the right.

A few days after admission a craniotomy was performed and the left frontal lobe explored. No tumor was encountered. Subsequent to the operation hyperpyrexia and circulatory instability developed, and the patient died the following day.

Autopsy.—The brain revealed asymmetry due to an increase in the size of the frontal lobe of the left hemisphere. In this region there was an operative cavity, measuring 4 by 5 cm. Coronal sections of the left cerebral hemisphere revealed 2 tumors. One was near the frontal pole and the other in the temporal lobe (fig. 1 *A* and *B*). Both were of the same appearance and consistency but did not seem to be united at any point. On cut section they were hemorrhagic and necrotic, but not encapsulated, and had the characteristic gross appearance of gliomas.

The tumor in the frontal lobe was situated immediately beneath the operative cavity. It measured 3 by 2 cm. in the vertical plane and encroached on the inferior surface of the frontal lobe but was separated from the surface by a thin layer of cortex (fig. 1 *A*). The tumor extended posteriorly along the frontal horn of the lateral ventricle for a distance of 3 cm.

The other tumor, in the posterior part of the left temporal lobe, was likewise separated from the surface by a thin layer of cortex. Beneath the surface it measured 4 by 3 cm. and extended to a depth of 3 cm. (fig. 1 *B*). Both tumors merged imperceptibly into the surrounding brain tissue and were entirely intracerebral. There was no attachment to the dura.

The only abnormalities observed on cut section of the right cerebral hemisphere were generalized thinning of the cortex, numerous subependymal petechiae and a large clot filling the frontal pole of the lateral ventricle. There was no hydrocephalus. The basal structures and cerebellum were uninvolved.

Microscopic Appearance.—The tumors were similar in histologic appearance. For purposes of description they can be considered as divided into three zones—central, middle and peripheral. The large central portion was composed of a necrotic hemorrhagic material. In this area, thickened and degenerated walls marked the outline of many former blood vessels. The middle zone was made up of actively proliferating fibroblastic tissue and was very cellular, with an irregular arrangement (fig. 2 *A* and *B*). Intercellular collagenous fibers, which stained blue with the azocarmine, were numerous; they appeared in large sheets between the proliferating cells. Nerve and glial fibers, which showed degenerative changes, had been entrapped by this rapidly growing tissue. Blood vessels were conspicuous and appeared to be a part of the neoplastic process, if not the actual

source of it (fig. 2 *B*). The peripheral zone of the tumor represented the neuroglial reaction to the invasion of the fibroblastic elements. Here there was gradual blending of the reactive into the normal brain substance, with no sign of capsule formation.

The most characteristic part of each tumor was found in the middle zone, where one could study the variable structure of the proliferating tumor cells. The small spindle-shaped cells contained hyperchromatic nuclei. Their cytoplasm was abundant, streaming from either pole, and took a dull brick color. The intercellular fibers of such cells were few and were arranged in parallel fashion as though they



Fig. 1.—*A*, coronal section through the left frontal lobe showing the hemorrhagic, necrotic intracerebral tumor. *B*, section through the temporal neoplasm. This tumor is similar in appearance to *A* but is even less circumscribed and merges into the surrounding tissue.

had a definite objective. Mitotic figures were frequent in these areas (fig. 2 *A*). Other cells possessed large oval vesicular nuclei with a heavy nuclear membrane and clumped chromatin (fig. 2 *B*). Here the scanty cytoplasm was stellate. The fibers were more numerous and intertwined in aimless fashion to produce a reticular pattern. Intermediary forms, of course, were present. There was very little hyaline degeneration. Other cellular elements of connective tissue were also seen—macrophages, eosinophils and plasma cells.

The compressed and degenerated brain substance which lay adjacent to the tumors introduced a variety of cell reactions which slightly complicated the picture.

In this peripheral zone the gemistocytic astrocytes were more abundant than the pilocytic ones. These cells were made more conspicuous because of the partial demyelination of the surrounding brain tissue. Microglia cells were interspersed with the other neuroglial elements but were most numerous in the perivascular

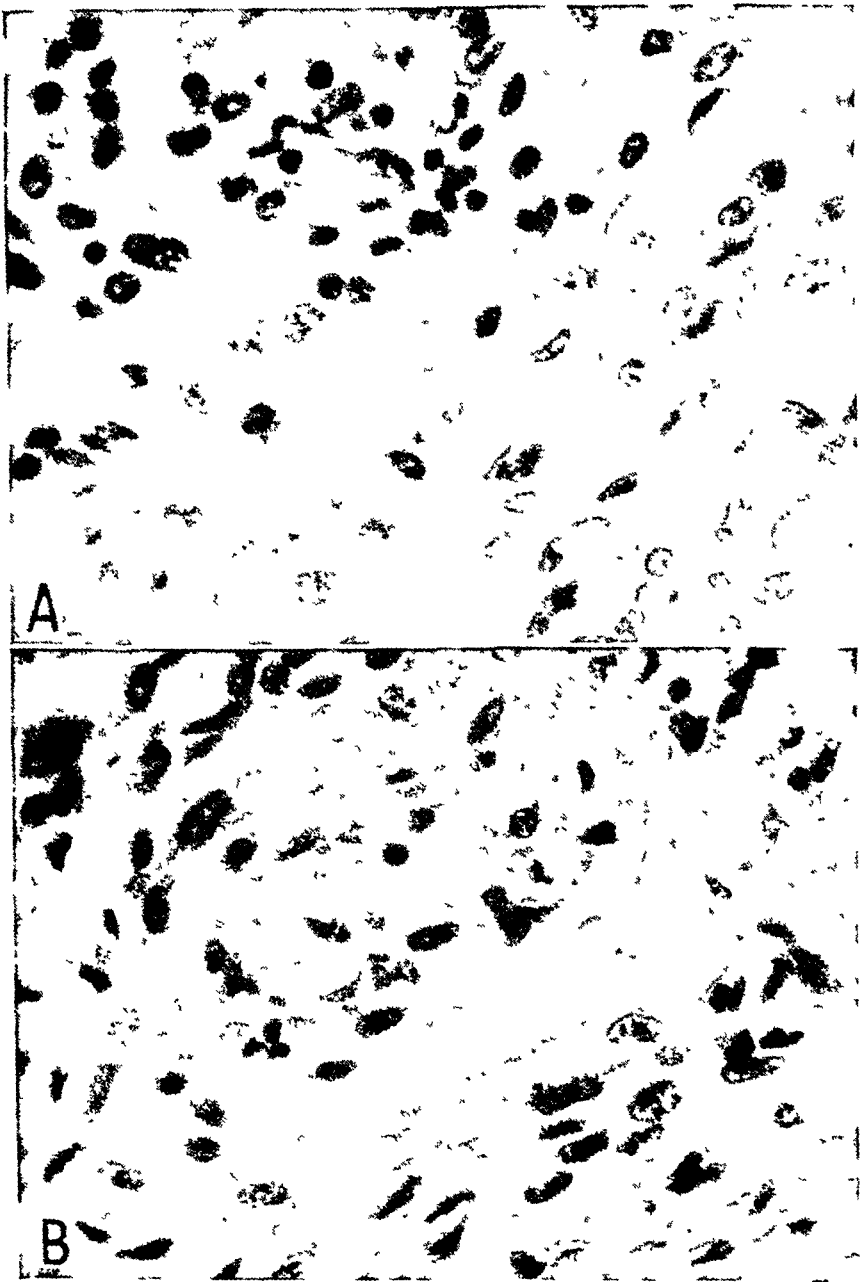


Fig 2—*A*, section through the cellular portion of the frontal tumor. Note the variable structure of the proliferating tumor cells. Mitoses are numerous. *B*, photomicrograph of the vicinity of a small blood vessel. The fibroblastic proliferation can be seen extending from the vicinity of the vascular adventitia into the surrounding tumor tissue.

spaces, where they had migrated with their ingested cerebral debris. Lymphocytes, polymorphonuclears and plasma cells could also be found in the perivascular regions, intermixed with the scavenger cells.

Throughout the tumors the blood vessels were increased in number, and their walls, in thickness. In the area of neuroglial reaction (peripheral zone) fibroblastic proliferation was seen in the outer layer of many of the vessel walls. Where the vessel consisted of a single layer of endothelium, a fibroblastic meshwork surrounded the vessel, enclosed the perivascular cells and extended into the adjacent brain tissue.

In the actively neoplastic zone one could observe large strands of connective tissue extending from the vicinity of the vascular adventitia into the surrounding tumor tissue (fig. 2*B*). This proliferating connective tissue had caused a distortion of the architecture of the vessels and a constriction of their lumens. The resultant thrombosis led to the necrosis which comprised the bulk of the tumor. The fibroblastic tissue which surrounded the still patent blood vessels was similar to that of the remainder of the tumor but was more dense and concentrically arranged. The density and nature of the fibroblastic growth prevented any perivascular hemorrhage around these vessels.

In summary, these neoplasms were considered to have had their origin in connective tissue because the type cell was the fibroblast. The neoplastic tissue was classed as sarcoma because of its invasive properties and because of the evidence of rapid growth. There was no sign of capsule formation. Many areas contained spindle-shaped cells with hyperchromatic nuclei and abundant cytoplasm. Intercellular substance was scanty in these areas, and mitotic figures were common. Centers of growth around blood vessels were a characteristic feature.

COMMENT

The 7 primary brain tumors of fibroblastic origin can be subdivided into 4 fibrosarcomas, 2 fibroblastomas and 1 fibromyxoma. They occurred in the frontal and temporal regions in patients ranging in age from birth to the age of 52 years. One tumor had a connection with the meninges.

Such tumors may have as their origin the pia, the vascular adventitia or the pia surrounding the blood vessels. Alpers, Yaskin and Grant⁴ stated, "There can be little doubt . . . that around the many vessels in our tumor, were tumor cells that arrange themselves in intimate relations to the vessels. They seem to be part and parcel of vessel wall." These authors suggested as an etiologic possibility those cells which, like the capillaries, are derived from embryonic connective tissue and which accompany the capillaries. The cells which accompany the capillaries were formerly called perithelium, but according to Maximow⁶ this term should be discarded as it included several types of cells. He believed that as the vessel becomes more complex the adventitia gradually merges with this cellular element and that undoubtedly both have a common source. Alpers, Yaskin and Grant⁴ concluded that the fibroblastic origin is from the leptomeninges, cerebral vessels or cells associated with vessels capable of differentiating into fibroblastic cells.

Meyer and Scheller⁵ offered, as the source of their fibromyxoma, mesenchymal rests or the adventitia of the blood vessels of the brain. The deformity of the skull since birth and the concomitant existence of lipomas of the leptomeninges and cysts of the kidney suggest the mesenchymal rest.

6. Maximow, A. A., and Bloom, W.: *A Textbook of Histology*, ed. 2, Philadelphia, W. B. Saunders Company, 1934, p. 245.

Although the derivation of the leptomeninges is still unsolved, it is known that they become invaginated to form the perivascular spaces of Virchow and Robin. Because of this close proximity to the vascular adventitia it is difficult to determine absolutely whether these tumor cells originate from the leptomeninges or from the vascular adventitia. In the present case tumor cells were found around capillaries and could well be derived from the undifferentiated mesenchymal cells. In vessels having more complex walls the proliferation apparently was occurring in the adventitia and spreading peripherally. The histologic evidence certainly favors the opinion that the tumor cells were arising from the connective tissue of the adventitia or from its precursor, the less differentiated mesenchymal cells.

SUMMARY

A case is reported in which 2 separate fibrosarcomas were found in the left cerebral hemisphere of a 52 year old man.

From the histologic studies of fibroblastic tumors of the brain it appears that their most probable origin is from the connective tissue of the adventitia or from its precursor, the less differentiated mesenchymal cells.

HEMOBLASTIC SARCOMA (PRIMITIVE RED CELL TYPE) FOLLOWING POLYCYTHEMIA VERA

DAVID PERLA, M.D., AND S. B. BILLER, M.D., NEW YORK

The case to be presented is a remarkable instance of overcompensation of hemoblastic tissue following the exhaustion of abnormal erythropoiesis, which was terminated by vigorous benzene and roentgen ray treatment.

REPORT OF CASE

A woman of 47 years was admitted to the hospital April 26, 1936. Seventeen years earlier, at the age of 30, her red cell count was 11,000,000, and a diagnosis of polycythemia vera was made. She was treated during a period of thirteen years with roentgen radiation and benzene. The treatment was discontinued in 1932, when the hemoglobin content dropped to 70 per cent and the red cells to 3,500,000. Five years prior to admission she had a series of attacks of chills and fever associated with herpes zoster and Horner's syndrome on the left side. These attacks were never explained. During the two years prior to admission her symptoms had been largely those of progressive severe anemia. She had been given repeated transfusions of blood and parenteral injections of liver. Six months prior to admission she began to have painful swelling in the region of the right scapula, which on biopsy was diagnosed as sarcoma of the scapula. This mass almost disappeared with roentgen therapy. A pleural effusion occurred on the left side, and a smear of sediment from the fluid revealed 23 per cent myeloblasts. At this time a count made on the peripheral blood showed, in addition to the secondary anemia, 14 per cent myeloblasts and 6 per cent normoblasts.

During the first two months of the patient's stay in the hospital numerous lumps developed over the jaw, sternum, abdomen and groin. They were painful and tender. These masses appeared at sites of injection or of injury.

On examination during her stay in the hospital, she presented an enlarged liver, a hard, firm enlarged spleen, an inconstant soft systolic murmur at the apex of the heart, signs of consolidation at the base of the left lung and an old unhealed abscess of the left buttock. In addition, masses of soft tissue were felt at the angle of the jaw on the left, over the right temporal region, at the left corner of the mouth, over the sternum, in the abdominal wall and in the right and left groins. The inguinal and the anterior cervical lymph nodes were enlarged, some being as large as a lemon.

The red blood cell count was 3,000,000; the hemoglobin content, 58 per cent; the color index, about 1. The white blood cell count was 3,100, with differential percentages as follows: segmental polymorphonuclears, 44; staff polymorphonuclears, 5; myelocytes, 2; myeloblasts, 22; lymphocytes, 19, and monocytes 7. The platelet count was 75,000. The bleeding, coagulation and clot retraction times were normal. Chemical examination of the blood revealed a low level of

From the Laboratory Division, Montefiore Hospital.

cholesterol, 125 mg.; calcium, 12 mg.; phosphorus, 3.7 mg.; protein, 4.1 Gm.; albumin, 2.2 Gm.; globulin, 1.9 Gm.; icterus index, 4; bilirubin, 0.3 mg.; sugar, 98 mg.; urea nitrogen 12 mg. The tourniquet test was negative. The blood plasma volume was slightly increased.

The gastric juice revealed achlorhydria even after administration of histamine. Bence Jones protein was not present in the urine. The basal metabolic rate was +64 and +57 per cent. The Wassermann and Kahn reactions were negative.

Roentgen examination revealed pleural masses, especially over the upper lobe of the left lung, and increase in the width of the superior mediastinum. A large mass of soft tissue was also seen involving the sternum. There was slight osteoporosis of the distal ends of the shaft of both femurs and of the lower third of the right tibia, but it was not characteristic of a destructive lesion.

Biopsy of the soft tissue mass in the abdominal wall revealed an appearance of chronic granulomatous tissue, which at the time was considered to be suggestive of Hodgkin's disease. The clinical diagnosis was myelosarcoma with the blood changes of myeloblastic leukemia.

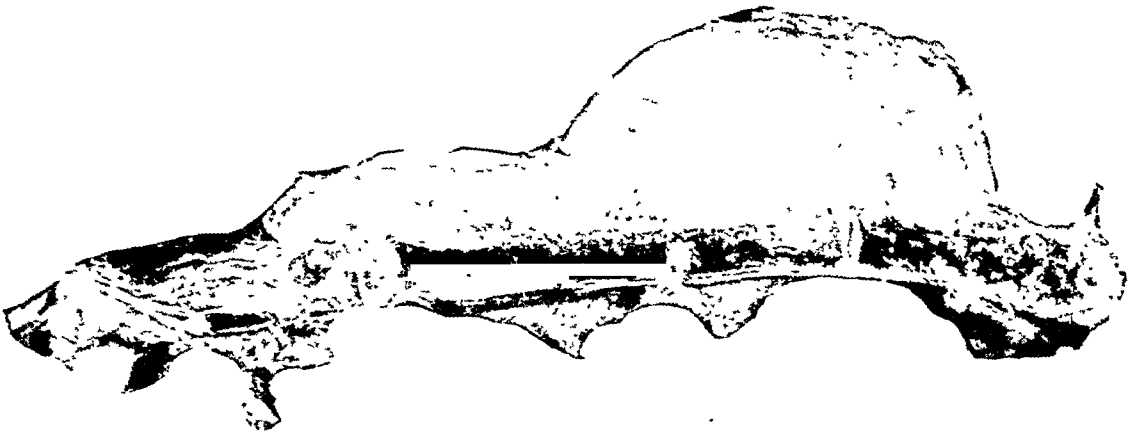


Fig. 1.—Cross section through the sternal mass.

The patient was extremely weak and declined rapidly. She died on June 1, five weeks after admission.

Autopsy.—The anatomic diagnosis was: hemoblastic sarcoma of a primitive red cell type, with invasion of subcutaneous and soft tissues, muscles, right femur, sternum, intercostal tissue, on the left vertebral column, pleura, lungs, left side of the diaphragm, heart, right kidney, gastrointestinal tract, left inguinal nodes, tonsils and uterus; diffuse hemosiderosis; splenomegaly; indeterminate endocarditis of the mitral valve, and an unhealed gluteal abscess on the left.

A large tumor mass, 9 by 6.5 by 5 cm., was present over the greater portion of the sternum. This mass was firmly adherent to the overlying skin and also to the underlying bone. On section it revealed erosion of the bony structure. It was partly necrotic but generally firm and whitish pink. Subcutaneous nodules were encountered in the scalp and left cheek, over both mandibles, in the right side of the face just anterior to the ear and in the left breast. They varied in diameter from 1 to 6 cm. On section they resembled the mass over the sternum. An abscess was present in the wall of the right upper quadrant of the abdomen. On section an extensive hemorrhage was encountered in the surrounding muscle

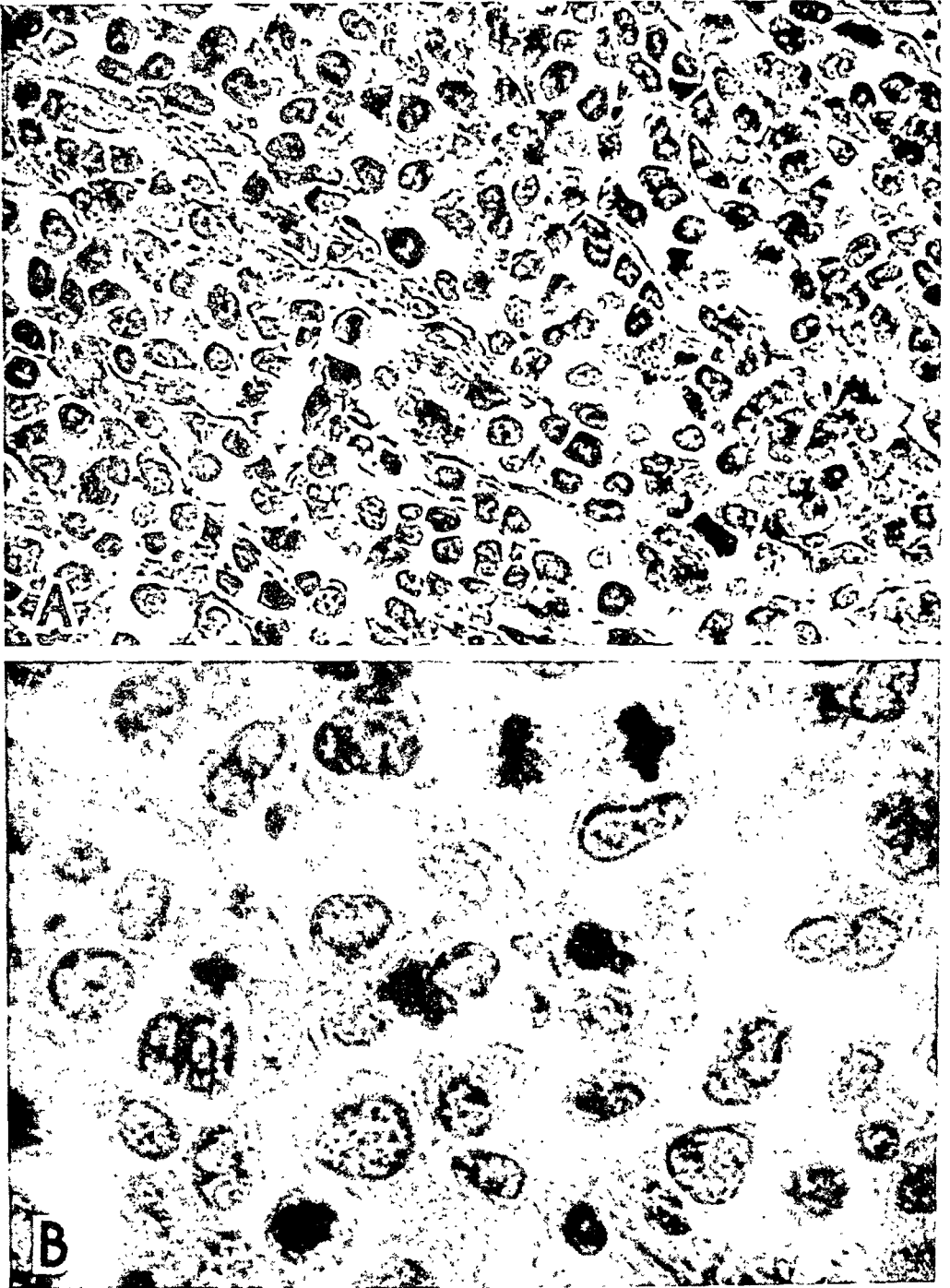


Fig. 2.—Microscopic appearance of the sternal tumor mass. Note the large numbers of stem cells. *A*, $\times 240$; *B*, $\times 1,200$.

tissue. A large abscess mass in the buttock contained tumor tissue in the surrounding muscle. A series of matted firm enlarged lymph glands filled the entire right axilla and extended down to the scapula. There were similar nodes in the left inguinal region. Tumor tissue infiltrated all the anterior mediastinal soft tissues and both tonsils.

The major vessels coming from the arch of the aorta were surrounded by tumor tissue. The left pleural cavity was obliterated by adhesions and extensively infiltrated by tumor, particularly at the base. The major portion of the left parietal pleura and the diaphragm were infiltrated and studded throughout with firm pink-white tumor nodules. On section the right lung contained one firm area of tumor tissue close to the hilus. Subpleural nodules were present throughout both lungs.

The spleen was enlarged (725 Gm.) and firm. On section the surface appeared somewhat glazed and pinkish red. The markings were not distinct. There was a small nodule, partly necrotic, beneath the capsule.

In the fundus of the stomach, along the greater curvature, was a large raised circular nodule, 5 by 5 by 1 cm., which on section contained pink-white tissue. Similar nodules were found beneath the mucosa of the cecum, colon and rectum, also in the substance of the right kidney and in the body of the uterus. They varied in diameter from 1 to 4 cm. The paravertebral muscles and soft tissue were infiltrated by tumor. The marrow of the vertebrae was deep red and hyperplastic. The thoracic vertebrae showed areas of firm pink-white tumor. The marrow of the right femur was completely replaced by tumor.

Microscopic Examination.—The tumor nodules and bone marrow were similar in appearance. In the marrow of the right femur the cells were arranged in solid sheets, which were apparently confluent hemopoietic nodules, the most primitive cells of which were centrally located. The large predominant cell measured about 20 microns in diameter, was rounded or oval and contained nongranular basophilic cytoplasm, occasionally vacuolated. The cell membrane was indistinct. The large nucleus, rounded or oval, was frequently notched in kidney bean fashion; it filled most of the area of the cell and was slightly eccentric. The nuclear membrane was coarse and sharply defined. Many dense nucleolar bodies were irregularly scattered throughout the nucleus. The nucleus was frequently seen in mitotic division. These cells were interpreted as hemocytoblasts.

More peripherally there were slightly smaller spherical cells with round, regular nuclei and more or less evenly distributed nucleoli, suggestive of proerythroblasts and erythroblasts. Adjacent to these were many smaller cells, about 9 microns in diameter, with small round nuclei containing dense chromatin material. The cytoplasm was basophilic in some and eosinophilic in others. The appearance was characteristic of normoblasts in various stages of development.

Occasional large cells, similar in appearance to the hemocytoblasts, contained eosinophilic granules in their cytoplasm; some revealed horseshoe-shaped nuclei. These were interpreted as promyelocytes and myelocytes.

Some cells, about twice the size of the hemocytoblasts, with irregular cytoplasmic projections and large dense irregular nuclei, appeared to have the characteristics of megakaryocytes.

The mass in the sternum, as well as other masses, consisted of primitive hematopoietic elements, chiefly hemocytoblasts and many erythroblasts and normoblasts, arranged in a fashion similar to that seen in the bone marrow. Hemorrhage, necrosis and old blood pigment were scattered throughout.

COMMENT

This condition was interpreted as a stem cell sarcoma of a primitive red cell type. It was an interesting example of overcompensation following exhaustion of erythropoiesis by benzene and roentgen therapy, instituted for the treatment of polycythemia vera. The hemoblastic sarcoma of a primitive red cell type may be looked on as a phase of overcompensation following this exhaustion and may be operative through a central mechanism. So-called myelogenous leukemia has been known to occur as a late phase in cases of exhausted polycythemia vera, but this seems to be the only instance on record, to our knowledge, of a tumor of a primitive red cell type in a human being.

BALL THROMBUS OF THE HEART

Report of a Case with Review of the Literature

C. G. ARONSTEIN, M.D.

Associate Pathologist, Providence Hospital

AND

LESTER NEUMAN, M.D.

Pathologist, Providence Hospital

WASHINGTON, D. C.

Up to 1924 Abramson¹ was able to collect from the literature only 22 cases of ball thrombus of the heart, to which he added the twenty-third. His article is excellent and should be read by every one interested in this condition. Since then, other cases have been reported,² bringing the total to 30 cases. Ball thrombus of the heart is therefore of sufficient rarity to warrant report of another case.

As the term is used in this paper, a ball thrombus must fulfil the criteria set down by Welch;³ i. e., there must be (*a*) entire absence of attachment, with consequent free motility; (*b*) imprisonment in consequence of an excess in the diameter of the thrombus over that of the first narrowing in the circulatory passage ahead of it, and (*c*) such consistency and shape that the thrombus will not of necessity lodge as an embolus in the passage. Separation of cases of this particular type of thrombus into a group is of somewhat academic interest since, clinically, similar symptoms may be produced by a pedunculated thrombus⁴ of the heart, as well as by a large vegetation of subacute bacterial endocarditis protruding into the mitral orifice (Schiller;⁵ Schwartz and Biloon^{2e}). However, these cases do seem to fall into a group by themselves, and probably the condition deserves to be considered as a pathologic entity.

REPORT OF CASE

A white woman 43 years of age was admitted Aug. 18, 1937, complaining of spells of vomiting after meals. The attack for which she was admitted had begun

From the Pathologic Laboratory of Providence Hospital.

1. Abramson, J. L.: *Ann. Clin. Med.* **3**:327, 1924.

2. (*a*) Cleland, J. B.: *M. J. Australia* **2**:50, 1936. (*b*) Covey, G. S.; Cook, R., and Rogers, F. L.: *Am. J. M. Sc.* **175**:60, 1928. (*c*) Elson, J.: *Am. Heart J.* **10**:120, 1934. (*d*) Potter, E. B.: *Ann. Clin. Med.* **4**:736, 1936. (*e*) Schwartz, S. P., and Biloon, S.: *Am. Heart J.* **7**:84, 1931.

3. Welch, W. H., in Allbutt, F. C.: *A System of Medicine*, London, Macmillan & Co., 1899, vol. 6, p. 185.

4. Kaplan, D., and Hollingsworth, E. W.: *J. A. M. A.* **105**:1264, 1935.

5. Schiller, I. A.: *J. Mt. Sinai Hosp.* **2**:153, 1935.

two weeks previously. Nausea did not always precede these attacks. There was no pain associated with the vomiting at any time. Such spells had occurred at intervals of several weeks to a year for the past five years, each lasting about a week. One month before admission a cough developed, with expectoration, which gradually lessened until it disappeared two weeks before admission. There had been a loss of 20 pounds (9.1 Kg.) in the past two years. During the past few years there had been irregular attacks of asthma at any time of the year, which the patient associated with taking cold. The menstrual history was entirely normal. The patient had a child, living and well, 17 years old. The past medical history revealed: chickenpox, pertussis, measles and diphtheria. The family history was negative.

The patient was a rather emaciated white woman, who did not appear acutely ill. The head and neck were normal. The eyes reacted to light and accommodation. The throat was normal. The teeth were in fair condition, with several missing. The lungs were clear. As regards the heart, there was splitting of the first sound with frequent extrasystoles. There was slight enlargement of the heart. There was a systolic murmur over the entire precordium. A faint pre-systolic or a late diastolic murmur was also believed to be present. The blood pressure was 120 systolic and 80 diastolic; the pulse rate, 68. The abdomen was somewhat retracted, and the liver was considerably enlarged but not tender. The diagnosis was rheumatic valvulitis with mitral stenosis and chronic gastritis.

The hemoglobin content was 68 per cent (Dare); the red blood cell count, 3,500,000; the white blood cell count, 11,000, with 87 per cent polymorphonuclears, 11 per cent large lymphocytes and 2 per cent small lymphocytes. The Wassermann and Kahn tests were negative. The Van den Bergh direct reaction was negative; the indirect reaction, less than 0.3 mg. per hundred cubic centimeters.

Ten days after admission, the pulse rate began to rise and remained high, varying between 90 and 130 per minute. During the first five or six days the patient was slightly febrile, the temperature never rising over 100 F. After this, but for one slight rise to 99.6 F. on the thirteenth day of illness the temperature remained normal until the last nine days of life, when it became subnormal. The patient became progressively weaker; transfusion did not alter the course of the illness. September 15 the patient died, after two days of marked dyspnea and cyanosis of the lips and face.

Postmortem Examination.—The body was that of a white woman in the fifth decade of life, small of stature, fairly well developed and showing emaciation of grade 3. The pupils were equal and slightly dilated. The abdomen was distended. The subcutaneous fat was fairly well preserved. The margin of the liver was 10 cm. below the ribs in the right midclavicular line and 20 cm. below the xiphoid process, being displaced downward by a collection of fluid in the right pleural cavity. The right pleural cavity was filled with a slightly cloudy olive green fluid, estimated at 1.5 liters.

The heart was enlarged, especially to the left, the apex reaching the mid-axillary line. The surface of the heart was smooth and glistening throughout. The tricuspid orifice admitted four fingers. The right ventricle showed definite hypertrophy. In handling the heart, before the left side was opened, a ball-like structure slipped out of the left atrium through the opening of an especially large pulmonary vein (fig. 1 *A* and *B*). This mass was almost perfectly round, 3.5 cm. in diameter, and showed no evidence of a pedicle or of a previous site of attachment. Its surface was covered with pinpoint-sized elevations, resembling "goose pimples," and the color of the mass varied from yellowish to deep rose.

It had evidently been lying within the left atrium as a free body. When the left atrium was opened, two masses of similar tissue were seen adhering flatly to the inner surface of this chamber, close to the mitral orifice (fig. 2). These masses were both irregular in shape; one was slightly triangular, measuring 2 by 1.5 by 1.5 cm. and having a thickness of about 1 mm., and the other was rather square, measuring 2.5 by 2.5 cm. and having a thickness of 2 mm. The latter could be easily dislodged. Both of these structures were covered by the same pinpoint-like elevations as the ball thrombus. The mitral valve was the seat of marked chronic fibrosis, due to an old rheumatic infection, which had resulted in a typical

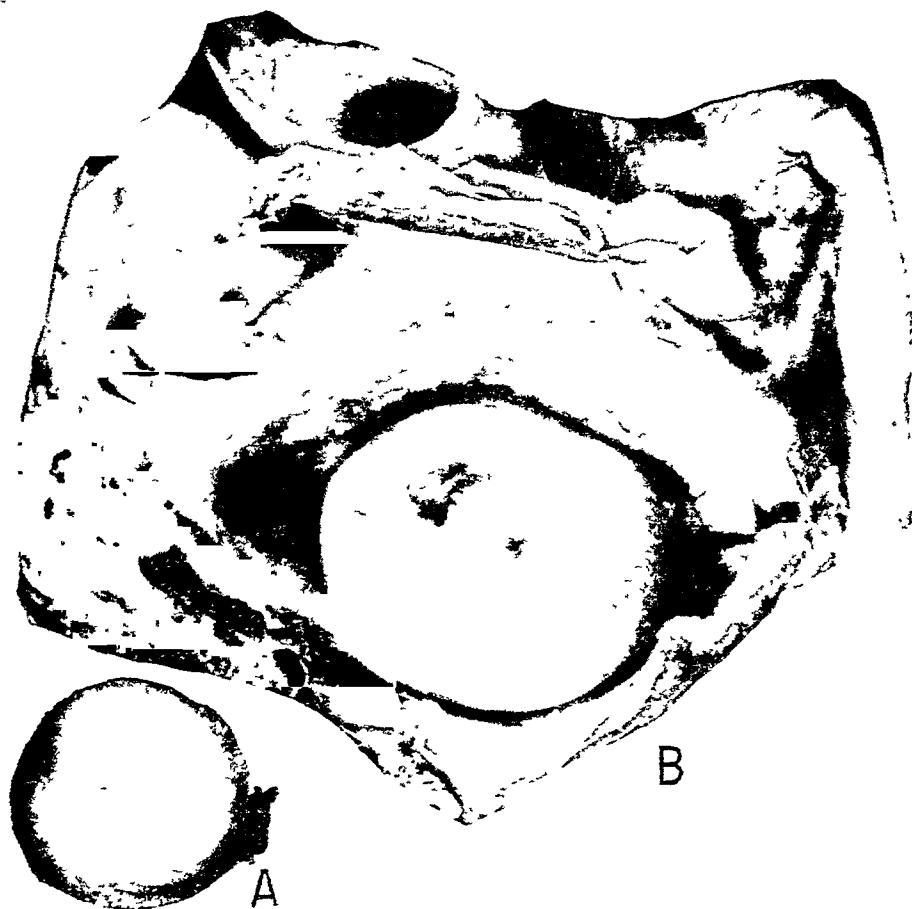


Fig. 1.—*A*, ball thrombus: Note the small pimple-like elevations. The rough area on the side is not a pedicle but an area in which the superficial layer of fibrin has become loosened from the mass. (The photographs are presented by permission of Lieut. Col. J. E. Ash, curator of the United States Army Medical Museum.) *B*, ball thrombus in situ, showing how completely the thrombus could fill the mitral orifice.

funnel-shaped valve that would not admit the tip of a finger. The greatest diameter of the opening was about 1 cm. (fig. 2). The aortic valve was normal, but the aorta itself was markedly hypoplastic, admitting only the little finger. The left ventricle showed no hypertrophy and seemed rather small.

The right lung was somewhat compressed by the collection of fluid referred to. However, on section the tissue was pale pink and crepitant throughout. The left

lung showed in the lower portion of the lower lobe a rather large area of increased density, which did not crepitate. On section this tissue showed a pale red area which was irregular, depressed and surrounded by very firm and moderately congested tissue. The upper lobe was crepitant throughout and on section was pale pink.

The spleen was bound down by dense adhesions. It was slightly enlarged, and the lower two thirds was the seat of an extensive degenerated infarct. The



Fig. 2.—Mitral valve. There is marked stenosis. The slitlike orifice is not clearly seen but was about 1 cm. in length.

upper pole contained another infarction, which was small, close to the periphery and surrounded by firm and markedly congested tissue.

The liver was normal in size. On section it showed the characteristic nutmeg appearance and was congested (grade 3). The gallbladder was considerably thickened and contained a small amount of dark green bile. There were no stones. The pancreas was normal in size but showed slight congestion. The adrenals were slightly pale but otherwise normal.

The left kidney was normal in size (175 Gm.). On section the cortex was of average thickness. The medulla showed numerous small cystic excavations. The outermost portion of the cortex showed a peculiar deposit arranged in linear

streaking, suggestive of calcium deposits. The right kidney was replaced by a small cystic mass, 3 cm. in greatest diameter, which on section showed two small thin-walled cysts filled with clear fluid.

The uterus was atrophic and contained no tumors. The fallopian tubes were thin walled and congested. The ovaries were markedly calcified and slightly increased in size.

Pathologic Diagnosis.—The following conditions were diagnosed: rheumatic heart disease with marked mitral stenosis; ball thrombus of the left atrium; cystic degeneration of the right kidney; splenic infarction; pneumonitis of the lower lobe of the left lung; chronic cholecystitis; sclerosis of the ovaries.

Microscopic Examination.—The examination showed slight calcium deposits in the renal tubules, fibrosis of the lungs with exudation of leukocytes into many alveoli and a few areas of perivascular fibrosis in the heart. The thrombus was not sectioned, as it was desired to preserve it intact. In previous cases section showed the thrombus to be composed of layers of fibrin, at times with a gelatinous core.

A review of the literature reveals the following facts in relation to cases of this type: Of the 21 cases in which sex was recorded, 17 concerned females and 4 males. This is in keeping with the sex incidence of rheumatic infection in general. The reported ages of the patients ranged from 15 to 55 years. Among the females, the highest frequency was in the fifth decade. Among males, the age of greatest frequency varied from 16 to 28 years. The ball thrombus therefore caused earlier death in the male than in the female, probably because of the less sheltered life of the male. In every case mitral stenosis was present, and in most cases, to a marked degree. The ball thrombus was found in the left atrium in all but a single case.¹ In this case it was found in the right ventricle.

The most universal symptom was dyspnea, and in every case it was prominent. In most cases the shortness of breath was extreme, and in many cases it was the presenting symptom. Embolic phenomena were fairly frequent. In those cases in which an antemortem diagnosis was made (or considered) the most prominent clue to the diagnosis, according to Abramson,¹ was the transient interference with the peripheral circulation. This was stressed greatly by Battistini and by Bozzolo, who diagnosed the condition clinically, although in their particular cases the cardiac thrombi were pedunculated. This, however, does not destroy the value of their findings, as the signs and symptoms would be the same whether the thrombus was free or attached. In many cases a cold and mottled extremity, cadaveric in appearance, became entirely normal within twenty-four hours, or a pulse that was faintly perceptible or imperceptible returned to normal. Another rather frequent embolic phenomenon met with was hemiplegia occurring within one or two years of the fatal outcome. This was seen in 5 cases.¹

Apparently the rheumatic infection responsible for the mitral stenosis is responsible for the formation of the ball thrombus also. In many cases an area of localized thickening was found in the endocardium of the left atrium. This was an irregular elevated patch, rather densely adherent, which could easily have been the site of origin of the nucleus

of the ball thrombus. After separation from this site of origin, the thrombus could become spherical and coated with a smooth laminated covering of fibrin.

In reviewing the cases in the literature as to symptoms and diagnosis, we have included certain cases in which a typical ball thrombus was not present but a thrombus that simulated the ball type clinically. Such instances are those of pedunculated thrombus of the left atrium and also those of certain large vegetations seen in subacute bacterial endocarditis involving the mitral valve. Incidentally, one of the interesting features of ball thrombi is that they have thus far appeared *only* in association with mitral stenosis, while pedunculated thrombi have occurred with other conditions, as hypertension. Of all the thrombi reported, i. e., ball thrombi, pedunculated thrombi and large occluding vegetations, only 11 have been diagnosed clinically, while of the 30 ball thrombi only 4 have been diagnosed clinically. However, the diagnosis is becoming more frequent, most of the thrombi of this type which have been recorded having been diagnosed in the last decade.

So, in spite of the infrequency of the diagnosis in the past, in certain cases the possibility that this condition is present should be at least suspected. Thus, according to Battistini, quoted by Abramson,¹ "the diagnosis of thrombi of the left auricle is possible as formed by the symptomatology and reconstructed by my two cases, that is: (a) signs of mitral stenosis; (b) disturbance of the general circulation; (c) entire debility of the pulse; (d) presence of gangrene of the lower extremities; and also the practical importance of the pulse in the radial and other accessible peripheral arteries." Later Elson,^{2c} in presenting a new case, suspected before death, restated these criteria for the diagnosis as follows: "... diagnosis is frequently impossible, but it can sometimes be made on the basis of (a) long-standing mitral stenosis usually with auricular fibrillation, and (b) widespread and transitory disturbances in the peripheral circulation." In our case no suggestive symptoms were noted.

SUMMARY

Another case of ball thrombus of the left atrium is reported, with a review of the literature, showing this to be the thirty-first case reported. The condition is found only in association with long-standing mitral stenosis. It may be suspected in a case of long-standing mitral stenosis with or without auricular fibrillation when widespread and transitory disturbances in the peripheral circulation are found. Among the latter, a cadaveric coldness of the extremities is prominently mentioned.

General Reviews

COMPLEX INFECTIONS

RICHARD E. SHOPE, M.D.

PRINCETON, N. J.

Until quite recently each infectious disease was generally believed to have a single and entirely specific etiologic agent. In fact, such a conception formed the basis of Koch's postulates for the proof of the causal relationship of an infectious agent to a definite disease. Rivers¹ has on several occasions pointed out that Koch's postulates could not be strictly applied to diseases caused by filtrable viruses, though even here the spirit of the postulates could be fulfilled easily enough. Rivers has furthermore called attention to the fact that in certain diseases of complex etiologic background the rules set down by Koch were not applicable. It is with this group of diseases of complex cause that I wish to deal.

My reasons for choosing such a subject were twofold. First, it seemed that the topic might lend itself well to pointing out the fallacy of thinking of infectious diseases in the old terms of Koch's postulates as invariably due to single agents. And second, the diseases to be considered are probably largely unfamiliar to those whose main interests lie in human medicine. Thus the discussion should at least avoid the repetition of already familiar knowledge.

Throughout the present paper the expression "complex infection" will be used to denote an infectious disease in which more than one agent plays an essential causal role; the expression "etiologic complex," to denote collectively the agents causally involved. Only those diseases that have been reasonably proved to be of complex cause will be discussed. These bear neither relation nor similarity to what are commonly known in medicine as mixed or complicated infections: all occur under natural conditions as definite clinical entities.

The story of the development of knowledge concerning the complex infections is as exciting and intriguing as a detective yarn and equally full of fortuitous observations, obscure deductions and retrospective simplicity. In narrating this story I intend to consider each disease indi-

From the Rockefeller Institute for Medical Research.

The Middleton Goldsmith Lecture, read before the New York Pathological Society, Dec. 7, 1938.

1. Rivers, T. M.: J. A. M. A. **107**:206, 1936; J. Bact. **33**:1, 1937.

vidually and to discuss the various observations and clues leading to its detection as a complex infection, together with the theories as to how the paired causative agents act in producing the disease. To facilitate understanding of the plan of presentation to be followed, the complex infections of which I shall speak are listed here, with their causative agents.

Disease	Causative Agents
1. Blackhead of turkeys	<i>Histomonas meleagridis</i> + <i>Heterakis gallinae</i>
2. Rugose mosaic of potato	Mottle (X) virus + vein-banding (Y) virus
3. Streak of tomato	Mottle (X) virus + tobacco mosaic virus
4. Swine influenza	<i>Haemophilus influenzae suis</i> + virus of swine influenza
5. Type III coryza of fowl	<i>Haemophilus gallinarum</i> + coccobacilliform bodies
6. Tulip breaking	Tulip virus 1 + tulip virus 2
7. Infectious myxomatosis of rabbits	Fibroma virus + Berry-Dedrick factor

It is evident that these diseases are diverse both as to hosts and clinical types. Furthermore, the etiologic agents are a heterogeneous collection, ranging from viruses through bacteria to protozoa and worms.

BLACKHEAD OF TURKEYS

Blackhead has been defined as an infectious disease of turkeys and, to a lesser extent, of chickens, caused by *Histomonas meleagridis*. The pathologic alterations, which are limited mainly to the ceca and liver, are those of caseous necrosis. At autopsy the livers of affected birds are found to be markedly enlarged and spotted with multiple focal areas of necrosis ranging from pinpoint size to a centimeter or more in diameter. The cecal walls are thickened, and the cecal lumens are filled by plugs of necrotic caseous material. The mucosa and submucosa are inflamed or necrotic and desquamated. Histomonads are plentifully present in the cecal and hepatic lesions.

The disease was first seriously investigated by Theobald Smith, and in 1895 he described as the cause a protozoan parasite, since called *Histomonas meleagridis*.² The initial claim of this parasite to consideration as the etiologic agent was based entirely on the fact that it was uniformly present in the lesions.

2. Smith, T.: Investigations Concerning Infectious Diseases Among Poultry, Bulletin 8, United States Department of Agriculture, Bureau of Animal Industry, 1895.

In later years Smith and Graybill became interested in the mode of spread of the disease. There were a number of epidemiologic facts not clearly explicable on the basis of a pure *Histomonas meleagridis* infection. For instance, turkeys fed feces containing *Histomonas meleagridis* from infected or recovered birds not infrequently failed to acquire blackhead. Furthermore, though the histomonads were known to be relatively fragile parasites and to be incapable of survival for long in material discharged from the ceca, pens in which infected turkeys had been kept remained infective for young turkeys long after any histomonad should still survive. Because of such epidemiologic discrepancies Graybill and Smith suspected that an accessory agent might be concerned either in the transmission of *Histomonas meleagridis* or in favoring its invasion and multiplication in the turkey. In harmony with this hypothesis they introduced into their experiments the common cecal worm of poultry, *Heterakis gallinae*, as the possible associated factor.³

Young disease-free turkeys were fed feces from old turkeys of a flock known to be infected, and with these feces were mixed cultures of embryonated ova of *Heterakis gallinae*. Blackhead developed in the young turkeys and all died. In a subsequent experiment Graybill and Smith included four groups of young turkeys. One group was fed turkey feces alone, one group was fed turkey feces mixed with embryonated ova of *Heterakis gallinae*, a third group received ova alone, and the fourth group served as unfed controls. No clinically recognizable blackhead appeared in the control group or in the group fed turkey feces alone. In the two other groups, however, typical blackhead developed in all the birds. From this experiment it appeared that blackhead could be produced in turkeys merely by feeding them embryonated ova of *Heterakis gallinae*. Further experiments both with turkeys and chickens confirmed this observation. However, though the disease had apparently been induced by embryonated ova of *Heterakis* alone, at autopsy the lesions of the experimental birds were shown to contain *Histomonas meleagridis*. These observations led Graybill and Smith to the assumption that either their experimental turkeys and chickens had been healthy carriers of histomonads or their cultures of *Heterakis gallinae* had been contaminated by these parasites. They did not definitely choose between the two possibilities, though they favored the latter. At any event, they visualized the blackhead produced as resulting from the concerted activity of *Histomonas meleagridis* and *Heterakis gallinae*. Supposedly, the larvae of *Heterakis gallinae* prepared the way for the destructive invasion of the walls of the ceca and the liver by *Histomonas meleagridis*.

3. Graybill, H. W., and Smith, T.: J. Exper. Med. **31**:647, 1920. Smith, T., and Graybill, H. W.: *ibid.* **32**:143, 1920.

But it remained for Tyzzer^{4a} and Tyzzer and Fabyan^{4c} to demonstrate the actual cooperation by which *Histomonas meleagridis* and *Heterakis gallinae* produce blackhead. They experienced no difficulty in confirming the correctness of the observation of Graybill and Smith that embryonated ova of *Heterakis gallinae* frequently produce blackhead when fed to young disease-free turkeys. Furthermore, by using great care in hatching and rearing their experimental birds they procured young turkeys that were undoubtedly not carriers of *Histomonas meleagridis*. In such birds the embryonated ova of *Heterakis gallinae* still produced blackhead, and histomonads were present in the lesions. This made it seem quite clear that the ova had been contaminated by histomonads, but it did not indicate whether the contaminating protozoans were within the worm eggs or whether they merely adhered to the surfaces of the eggs. The question was finally answered by some extremely ingenious experiments. Instead of incubating the ova of *Heterakis gallinae* in the usual way, in salt solution, the cultures were prepared in 1.5 per cent nitric acid. This concentration of acid was sufficient to kill any adherent histomonads quite promptly, and such cultures after three days proved even bacteriologically sterile. It was clear that the procedure adequately eliminated histomonads that were lying external to the shells of the worm eggs. The ova were kept in the acid until they could be observed with the microscope to have become embryonated. They were then fed to young histomonad-free turkeys. Blackhead developed, and histomonads were observed in the lesions.

Even more conclusive were the results obtained by feeding samples of heterakid material, some before and some after the eggs had become embryonated and hence hatchable. In these experiments the nonembryonated ova failed to cause blackhead, while the embryonated ones induced the disease. Such results indicated that infection following the feeding of heterakid material was dependent on the hatching of the eggs of the worm in the alimentary tract of the bird. Strangely enough, Tyzzer was never able to see histomonads microscopically in demonstrably infective heterakid eggs though they have been seen in the gut of the newly hatched larva.

From the observations just summarized it seems clear that *Histomonas meleagridis* is carried within the eggs of *Heterakis gallinae* and persists in the eggs during embryonation even in 1.5 per cent nitric acid. When the embryonated ova hatch in the alimentary tracts of the turkeys to which they have been fed, the young larvae penetrate the epithelium of the cecal glands, doubtless carrying their histomonads with them. Here they increase in size, damaging and separating the epithelium by

4. (a) Tyzzer, E. E.: Proc. Soc. Exper. Biol. & Med. **23**:708, 1925-1926; (b) Proc. Am. Acad. Arts & Sc. **69**:189, 1934. (c) Tyzzer, E. E., and Fabyan, M.: J. Exper. Med. **35**:791, 1922.

pressure and eventually producing an inflammatory reaction. *Heterakis gallinae* thus not only serves as the intermediate host for *Histomonas meleagridis* but also provides it with a portal of entry by damaging tissues and preparing them for parasitization by this protozoan. It seems to me that in blackhead one has a rather good example of cooperative action by two agents in producing a disease: a truly complex infection.

Some objection to the classification of blackhead as a complex infection might be raised on the ground that, as Tyzzer and his co-workers showed, the disease can be produced by *Histomonas meleagridis* alone if the histomonads are actually inoculated into turkeys. For instance, if material containing the histomonads but free from *Heterakis* is administered to birds subcutaneously, intramuscularly, intravenously or by rectal inoculation, blackhead is usually produced. These routes of infection are, however, highly artificial, and it seems most likely that under natural conditions both *Histomonas meleagridis* and *Heterakis gallinae* are causally involved.

It may occur to some one that if blackhead is to be considered a complex infection on the grounds I have just outlined, then malaria, yellow fever and other diseases with insect intermediate hosts should be classified in the same way. It is clear as to these insect-transmitted diseases that no infection with the specific virus or parasite would take place were it not for the insect intermediary which carries the agent and prepares a portal of entry for it in much the same fashion as *Heterakis gallinae* effects an entrance for the histomonad of blackhead. To me the difference lies in the fact that the insect intermediate hosts are free-living forms, do not themselves invade the host's body and thus may not, broadly speaking, be classed as infectious agents. If hard pressed by argument I might admit that blackhead may be a borderline example of a complex infection and less definite than some of the other diseases which I wish to discuss.

RUGOSE MOSAIC OF POTATO

The situation regarding the exact etiologic explanation of the various virus diseases affecting the potato plant is admittedly difficult and confusing. This confusion, however, is not so much concerned with facts as with nomenclature, and it seems established that at least several of the diseases of potato plants are complex virus infections. Only one of these, rugose mosaic, will be discussed. This disease is one of the more serious of those affecting American potato plants. Plants with rugose mosaic frequently die before the production of tubers, or their tubers are reduced in size. The plants themselves are dwarfed and curled with rugose, abnormally hairy leaves. The lower

leaves generally have black necrotic veins, while the upper ones are mottled with light green spots.

A thorough understanding of rugose mosaic was initiated when Johnson⁵ demonstrated the presence of a virus in apparently healthy potatoes of most of the standard American varieties. This virus, seemingly completely innocuous for potato plants, induced either mottling or ringspot lesions when transferred to the leaves of tobacco plants. It was present, and readily demonstrable by the inoculation of tobacco plants, in both the foliage and tubers of the potato plants. Johnson considered two possible explanations for his observations: either the normal (or possibly abnormal) protoplasm of potatoes contained a substance capable of initiating a physiologic disturbance of tobacco and tomato plants which was of an infectious nature, or potatoes were almost universally infected with a virus. Subsequent work indicated that the second possibility was the correct one. The virus has been variously called potato virus X, mottle virus, healthy potato virus or latent potato virus.

Following the demonstration of the presence of X virus in most or all healthy potato plants, workers in three different laboratories discovered quite independently that this virus played a causal role in rugose mosaic.⁶ But associated with it in this disease was another virus, variously named potato virus Y, streak virus or vein-banding virus. Valteau and Johnson^{6a} in their experiments separated the viruses causing rugose mosaic, through the medium of nitrogen-starved tobacco plants. Smith,^{6c} on the other hand, broke up the virus complex by using what he termed plant indicators and plant filters, by taking advantage of selective insect transmission and by utilizing the unequal rates of movement of the constituent viruses in the infected hosts. Koch^{6b,d} utilized mainly selective insect transmission in his analytic experiments, and his data will be largely drawn on for the present discussion.

Prior to knowledge of the universal presence of potato X virus in healthy potato plants, Schultz and Folsom⁷ obtained apparent transmission of rugose mosaic from potato plant to potato plant by aphids. Koch readily confirmed this observation, using the aphids *Myzus persicae* and *Macrosiphum solanifolii*.

It was further known that when material from potato plants infected with rugose mosaic was transferred by inoculation to tobacco

5. Johnson, J.: Transmission of Viruses from Apparently Healthy Potatoes, Research Bulletin 63, University of Wisconsin, Agricultural Experiment Station, 1925.

6. (a) Valteau, W. D., and Johnson, E. M.: The Relation of Some Tobacco Viruses to Potato Degeneration, Bulletin 309, University of Kentucky, Agricultural Experiment Station, 1930. (b) Koch, K.: Science **73**:615, 1931. (c) Smith, K. M.: Proc. Roy. Soc., London, s.B **109**:215, 1931. (d) Koch, K.: Phytopathology **23**:319, 1933.

7. Schultz, E. S., and Folsom, D.: J. Agric. Research **25**:43, 1923.

plants symptoms of spot necrosis appeared on the tobacco leaves. When, however, aphids were used in attempting to transmit rugose mosaic from the potato to the tobacco plant, the lesions produced on the tobacco plants bore no resemblance to the spot necrosis caused by mechanical inoculation. Instead, the tobacco plant showed faint symptoms of quite another type—mainly, clearing along the leaf veins and general flattening of the plant. This mild disease transmitted to tobacco plants by aphids that had fed on potato plants infected with rugose mosaic proved readily transmissible in series in tobacco plants, and the agent responsible for it was shown to be a virus. Because of the character of the disease which this virus caused in tobacco plants it was called, by Valleau and Johnson, vein-banding virus, a name also adopted by Koch.

The discrepancy between the types of disease produced in tobacco plants by mechanical inoculation and aphid transmission suggested that two distinct viruses might be associated with the potato disease. From the experiments cited it appeared likely that both of these possible viruses were readily transmissible by artificial methods of inoculation, while only one of them, namely, the vein-banding virus, was transmissible by aphids. The nature of the other virus involved was suggested by the observation, mentioned earlier, that rugose mosaic was readily transmissible from potato plant to potato plant by aphids. Since these insects could apparently transmit the complete disease from potato plant to potato plant but only the vein-banding virus from potato plant to tobacco plant it seemed obvious that they were transmitting only vein-banding virus in both cases and that the hypothetical second virus must be one normally present in the potato plant. Knowledge of the ubiquity of Johnson's mottle, or potato X, virus in healthy potato plants suggested it as the second virus in the complex, and in a series of subsequent experiments the correctness of this suggestion was proved. When pure samples of mottle and of vein-banding virus were mixed and transferred to tobacco plants, symptoms of spot necrosis like that caused by rugose mosaic material resulted, though the individual viruses, inoculated separately, produced respectively only symptoms of mottle or of vein banding. Furthermore, apparently healthy potato plants known to be carriers of virus X showed rugose mosaic after inoculation with vein-banding virus alone. The experiment crucial to proof was done with seedling potatoes that were demonstrably free from virus X. In this experiment one group of seedling potatoes were inoculated with vein-banding virus alone, one group with a mixture of vein-banding and potato X virus and a third group with rugose mosaic material. The first group, receiving vein-banding virus alone, remained free from rugose mosaic, though a slight rugosity and mottling of the upper leaves occurred. The second and third groups, inoculated respectively with a mixture of vein-banding and X virus and with rugose

mosaic material, presented symptoms of rugose mosaic. Such experiments clearly demonstrated the complexity of the etiologic background in rugose mosaic of potato.

No explanation of the mechanism by which the two viruses supplement one another in rugose mosaic has been advanced, but it seems to be generally assumed that their destructive effect on the plant is an additive one. With the X virus as widely distributed in potato plants as it is in this country, only the vein-banding virus need be transmitted from plant to plant during an epiphytotic. Thus this particular disease, though it is in fact a complex infection, appears from an epidemiologic standpoint to be a simple infection, and, were it not for the universal presence of the symptomless infection with X virus, the changes produced would be so mild as to attract little attention. Rugose mosaic of potato serves as an admirable example of a severe and serious disease caused by the concerted activity of two mild infectious agents.

It is interesting to speculate on the state of knowledge had it happened that the potato X virus was strictly species specific and infectious only for the potato plant. Its presence would probably never have been detected, and rugose mosaic would have been considered, on all experimental grounds that could be applied, as a simple infection caused by the vein-banding virus alone.

STREAK OF TOMATO

The tomato plant has a number of virus diseases, both simple and complex, and I should like to discuss briefly one of the complex type which is known as streak. This is one of the more serious diseases of the tomato plant, though fortunately it is not extremely prevalent. The characteristic symptoms are necrotic lesions on stem, leaves and fruit. The lesions on the stem take the form of dark longitudinal streaks, and the stem itself is brittle and easily broken. The leaves show necrotic spots and patches which gradually enlarge, shriveling the leaves. On affected fruits rounded or irregular sunken blotches may occur. Young plants are usually killed by the disease.

Suspicion that tomato streak might be a complex infection seems to have been first aroused by the work of Johnson⁵ with the X virus of the healthy potato plant. During the course of that work he had occasion to inoculate tomato plants with a mixture of his potato X virus and tobacco mosaic virus. The resulting disease was much more severe than that caused by either virus alone and sometimes killed the plants. The following year Vanderpool⁸ made the significant observation that if streak material was dried from two to nine months and then inoculated into tomato plants it no longer produced streak but only symptoms of

8. Vanderpool, T. C.: *Phytopathology* **16**:311, 1926.

mosaic disease. He thus demonstrated that a strain of tobacco mosaic virus had been present in his original streak material but that the prolonged drying had destroyed some other factor essential to the production of streak. Acting on the suggestion furnished by Johnson's experiments, Vanderpool explored the possibility that the agent destroyed by prolonged drying of his streak material had been a potato virus. He inoculated tomato plants with a mixture of tobacco mosaic virus and potato mosaic virus and obtained what appeared to be typical streak. Stover⁹ showed that tomato streak was produced in plants inoculated with mixtures of tobacco mosaic virus and material from any one of a number of potato diseases as well as with juice from apparently healthy potato plants, while Blood¹⁰ induced streak in tomatoes by inoculating with mixtures of tobacco mosaic virus and juice from healthy potato plants. In view of the ubiquity of the healthy potato virus it seems entirely probable, as Valteau and Johnson^{11a} pointed out, that the potato plants used in all of the experiments cited contained the healthy potato virus.

In analyzing the etiologic data in two naturally occurring epiphytotics of tomato streak, Valteau and Johnson^{11b} and Jones¹² found that each was caused by the combined activity of healthy potato virus and tobacco mosaic virus. It seems established, then, that tomato streak is a complex infection in which the causative agents are X virus of potato and mosaic virus of tobacco. Either virus alone produces rather mild symptoms in the tomato plant; the combination causes a severe and frequently fatal disease, the symptoms of which are distinctive. The effect of the two viruses, as in the case of rugose mosaic of potato, is supposedly an additive one. The disease serves as an example of a complex infection in which both causative agents are normally parasitic on hosts other than the one they attack concertedly.

SWINE INFLUENZA

Swine influenza may be defined as a highly contagious acute respiratory disease caused by *H. influenzae suis* and the virus of swine influenza.

The clinical and pathologic features of the disease will be summarized briefly. Its onset is sudden and the morbidity in an affected herd high: practically all animals under 1 year of age become sick.

9. Stover, W. G.: *Phytopathology* **18**:154, 1928.

10. Blood, H. L.: *Phytopathology* **18**:311, 1928.

11. Valteau, W. D., and Johnson, E. M.: (a) *Phytopathology* **20**:831, 1930; (b) **21**:1087, 1931.

12. Jones, L. K.: *Phytopathology* **22**:999, 1932.

Fever, anorexia, prostration of an extreme type, cough, leukopenia and rapid respiration of a peculiar abdominal type are salient features.

The period of illness is short, varying from two to six days, and if the disease is uncomplicated recovery is almost as sudden as the onset. The mortality usually ranges from 1 to 4 per cent. Autopsy on from the third to the fifth day of illness reveals a mucopurulent bronchitis and bronchiolitis and an atelectatic pneumonia variable in both amount and distribution but not infrequently involving portions of as many as five of the seven lobes. The pneumonic areas are purplish red, depressed, firm and leathery, while the adjoining lung tissue is emphysematous, exaggerating the depressed appearance of the involved portions. In fatal cases there is, in addition, diffuse bloody pulmonary edema.

The late Dr. Paul Lewis and I began our work on swine influenza during the epizootic which occurred in the autumn of 1928. We obtained from Iowa two strains of infectious material in the form of diseased lung and bronchial exudate and both of these strains proved readily transmissible to experimental swine by nasal inoculation.¹³ The experimental disease faithfully reproduced that seen naturally in the field, and was maintained in swine by serial nasal passage at three or four day intervals or by contact in the pen. At autopsy the respiratory tracts of these experimental swine were studied bacteriologically in the hope that we might learn the cause of the disease. This bacteriologic investigation reached an exciting stage promptly, for from the first passage swine inoculated with each of our strains of swine influenza material there was isolated, in pure culture, an organism similar if not identical to Pfeiffer's *Haemophilus influenzae*. The same bacterium was isolated thereafter from all swine infected in later passages with either strain of the disease, provided they came to autopsy within seven days after the onset of fever. Frequently no organism other than this influenza bacillus-like bacterium could be recovered from the lungs or the bronchial exudate of infected animals. Because of the similarity of our bacterium to Pfeiffer's bacillus and because of its constant association with swine influenza we called it *Haemophilus influenzae suis*.¹⁴ Prejudiced perhaps, at the time, by our inclination to view Pfeiffer's bacillus as the most likely claimant for consideration as the cause of human influenza, we felt quite convinced from our findings with *H. influenzae suis* that this bacterium was the cause of swine influenza. In the numerous cases in which it was the only organism that could be isolated there was no choice but to consider it of etiologic importance, unless we wished entirely to deny it a role in the disease.

13. Shope, R. E.: *J. Exper. Med.* **54**:349, 1931.

14. Lewis, P. A., and Shope, R. E.: *J. Exper. Med.* **54**:361, 1931.

It was, of course, obvious that if *H. influenzae suis* was actually the cause of swine influenza it should fulfil Koch's postulates. The first pig inoculated intranasally with what we believed to be a pure culture became ill. The lesions produced were similar to those of swine influenza, and the organism was recovered in pure culture from the respiratory tract. This apparently positive result of our experiment with *H. influenzae suis*, coming when it did, was unfortunate, because it made us more certain than ever that we were dealing with the causative agent of swine influenza and quite effectively closed our minds for the time to other possibilities. The experiment was, of course, repeated in a second pig, but no illness resulted and at autopsy the animal was normal. Four other pigs inoculated intranasally with pure cultures likewise remained normal, and we began to wonder a little about our positive result. Even now, there is no certain explanation of that first experiment, provided indeed the animal had influenza as was believed at the time. We considered the possibility that *H. influenzae suis* might be one of those organisms that lose virulence rapidly when maintained on artificial culture mediums and spent the remainder of our first year trying to discover the cause of its loss of this hypothetical pathogenicity. All of these experiments gave negative results, which only supported our growing suspicion that *H. influenzae suis* was not the cause of swine influenza.

The following year four fresh epizootic strains of swine influenza were obtained from Iowa and transmitted to our experimental swine, and again *H. influenzae suis* was regularly encountered in animals ill of the experimental disease. In addition, the organism was isolated from 6 animals affected with the disease in the field—all that were studied bacteriologically. None of these newly isolated strains, however, possessed even the slightest pathogenicity for swine. In 1930 two new strains of swine influenza were obtained in Iowa. These proved readily transmissible, and again *H. influenzae suis* was the predominant or only organism that could be cultivated, but all efforts to produce the disease with the new cultures were unsuccessful. We were by now ready to abandon *H. influenzae suis* as the cause of swine influenza.

During the first year's work a few attempts to infect swine with sterile Berkefeld filtrates of known infectious material had been made. No illness remotely resembling swine influenza had resulted, and the results were considered negative. By 1930, when *H. influenzae suis* had failed so completely to fulfil the requirements of an etiologic agent, we were again ready to consider the question of a virus as the causative factor.

Swine were inoculated intranasally with Berkefeld V or N filtrates of lung and bronchial exudate suspensions, known to be infectious and

autopsies were made in four or five days. Of 10 experiments, 3 were interpreted as giving negative results, while in the remaining 7 some evidence was obtained that the injected filtrate had contained an infectious agent. The illness induced by this filtrable agent was definitely not swine influenza and—for want of a better name—was designated filtrate disease.¹⁵ Subsequent investigation has shown that the filtrable agent possesses all the properties requisite for classification as a virus.

Clinically the filtrate disease is much milder than swine influenza, and sometimes it is so ill defined that infections are difficult to recognize. In most cases there is no elevation in temperature, while in a few a fever for one day is observed. A moderate and transient apathy and some diminution in appetite are the usual symptoms shown. The extreme prostration and the various signs of extensive involvement of the respiratory tract so common in swine influenza are not seen.

At autopsy the lesions are slight as compared with the four and five lobe pneumonia of swine influenza. The lungs show only a scant, scattered, patchy lobular atelectasis, involving as a rule not more than small portions of one or two lobes.

After the establishment of the presence of a filtrable virus in swine influenza, the situation as to the cause of the disease itself became even more confused than it had been when *H. influenzae suis* was suspected. Here, instead of one agent that could be looked on as of possible etiologic importance, were two such agents. The bacterium could not be completely ignored, for while it had proved apparently perfectly harmless for swine, its constant presence in so many samples of infectious material from the field and its persistence on serial passage through experimental swine kept attention focused on it. Neither could the filtrable virus be accepted as the cause of the disease without reservation, because, while it unquestionably possessed pathogenic properties for swine, the mild illness that it caused was certainly not swine influenza. Considered in the light of the current conceptions of Koch's postulate that an infectious disease is caused by a single agent, it appeared that we had reached a point in our experiments where one too many were under suspicion. It seems obvious now that our data should at once have suggested that we were dealing with a complex infection. However, at the time that we were facing the incongruity of our results, the complex natures of the two plant diseases discussed earlier were not yet fully established, so that it was a little revolutionary even to consider the idea that two infectious agents might be required to cause a single infectious disease. But unlikely as the possibility seemed it was tested by inoculating a pig intranasally with a mixture of *H. influenzae suis* and the virus. The animal came down with swine influ-

15. Shope, R. E.: *J. Exper. Med.* **54**:373, 1931.

enza. With this lead a number of further experiments were carried out, and in these the effect of the virus alone and of the bacterium alone were carefully controlled. The results in all were the same: swine receiving *H. influenzae suis* alone remained normal clinically and were normal at autopsy; swine receiving virus alone acquired only the mild filtrate disease; those receiving mixtures of *H. influenzae suis* and the virus presented characteristic swine influenza. From these experiments it was evident that swine influenza is a complex infection caused by the concerted action of *H. influenzae suis* and swine influenza virus.¹⁵

The cooperative mechanism by which the two agents act in causing swine influenza is not definitely known. It seems likely, though, in view of the apparent complete harmlessness of the bacterium alone, that the virus, in damaging the respiratory epithelium as it does, creates both a portal of entry and a favorable medium for the bacterium. Endowed with the invasive assistance of the virus, *H. influenzae suis* probably behaves in the swine respiratory tract in much the same fashion as might another bacterium possessing invasive properties by its own right. That the assistance is not entirely one sided, however, is indicated by consideration of the histology of the disease. Lesions clearly bearing the imprint of virus activity are much more extensive in swine influenza than they are in filtrate disease, indicating that the pathogenicity of the virus is enhanced by the concomitant presence of the bacterium. From this it appears probable that swine influenza represents a synergistic complex infection in which each agent enhances in some way the pathogenicity of the other.

TYPE III CORYZA OF FOWL

Under natural conditions the domestic fowl is subject to an uncomplicated coryza, not unlike the common cold of man, in which the inflammatory reaction is limited to the mucosa of the nasal passages and the communicating portions of the orbital tract. A unilateral or bilateral mucopurulent nasal discharge is the salient observable feature. Nelson¹⁶ classified the disease into three types on the basis of length of incubation period and duration of coryzal signs. Type I has a short incubation period of from one to three days and a short duration of two weeks or less. Type II has a long incubation period of from nine to thirty days and a long duration of two months or more. Type III has a short incubation period, like type I, and a long duration, like type II. Early in his work Nelson suggested tentatively that coryza III was the basic form of the disease and that coryzas I and II were variants which tended to revert to it with continued passage through susceptible fowl. This suggestion was not entirely borne out when the causative

16. Nelson, J. B.: *J. Exper. Med.* 58:297, 1933.

agents of each type were finally determined. While only coryza III has proved to be a complex infection, it is necessary for clarity that the other two types be brought into the present discussion.

The causative agent of coryza I was the first to be discovered. It proved to be a hemophilic bacillus of very fastidious growth requirements¹⁷ and was named *Haemophilus gallinarum*. In pure cultures it reproduced quite faithfully the characteristic features of coryza I in fowl. *H. gallinarum* was also regularly present in the exudate from fowl with type III coryza, but pure cultures which had been isolated and transferred a few times on artificial mediums before testing in fowl failed to reproduce the type III disease. Instead they caused only coryza like type I. The relation of *H. gallinarum* to coryza of type III, with which it was always associated, was thus not very clearly defined. However, it was known that birds which had recovered from infection with *H. gallinarum* acquired coryza of slow onset and long duration when inoculated with exudate from fowl which had coryza III, and that *H. gallinarum* did not become established in their nasal passages. This modified coryza III in haemophilus-immune birds thus resembled coryza II both clinically and with respect to the uniform absence of *H. gallinarum*. Subsequently Nelson found that coryza II was caused by a minute gram-negative unclassified agent that he has termed the coccobacilliform bodies.¹⁸ The discovery of the cause of coryza II immediately suggested the possibility that coryza III might be a complex infection and have as its causative factors the agents separately responsible for coryzas I and II, namely, *H. gallinarum* and the coccobacilliform bodies. The first actual demonstration of the presence of the coccobacilliform bodies in coryza III was effected in a group of 5 birds that had recovered from infection with *H. gallinarum* and subsequently were inoculated with coryza III exudate.¹⁹ In these birds, after a long incubation period coryza like type II developed, and coccobacilliform bodies were demonstrated in all. From one of the birds the bodies were isolated in pure form in tissue culture. This strain, though deriving originally from coryza III material, was essentially the same as strains isolated from coryza II material.

It was now established that exudate from birds infected with coryza III contained both *H. gallinarum* and the coccobacilliform bodies. It could be demonstrated, moreover, that both agents were present throughout the entire course of the disease. This observation was contrary to the accustomed behavior of the two agents when injected in pure culture. The coccobacilliform bodies had seldom been demon-

17. Nelson, J. B.: *J. Exper. Med.* **58**:289, 1933; footnote 16.

18. Nelson, J. B.: *J. Exper. Med.* **63**:515, 1936; **64**:749 and 759, 1936.

19. Nelson, J. B.: *J. Exper. Med.* **67**:847, 1938.

strable before the tenth day, while *H. gallinarum* had seldom maintained an existence in the host for longer than two weeks.

The question of a complex etiologic background of type III coryza was finally settled by inoculating birds with mixtures of pure cultures of *H. gallinarum* and the coccobacilliform bodies. In these experiments the infectivity of each component alone was also controlled. In all the birds inoculated with the mixtures a type III coryza developed, of short incubation period and long duration, and both agents were regularly demonstrable throughout the course of the disease. In birds infected with the two components separately coryza I developed when they had received *H. gallinarum*, and coryza II when they had been given the coccobacilliform bodies. It is clear from the experiments outlined that the combined action of the two infective agents adequately accounts for the cause of type III coryza.

Nelson is of the opinion that *H. gallinarum* and the coccobacilliform bodies when present together in the nasal passages of the host cooperate in producing an effect that neither is able to accomplish alone. It is clearly a synergistic or, in the older sense (since both agents benefit by the association), a symbiotic reaction. The rapidly multiplying *H. gallinarum* creates a favorable environment for the immediate development of the coccobacilliform bodies. The latter, evidently by reason of their tendency to persist in the host, prolong the residence of *H. gallinarum*.¹⁹

TULIP BREAKING

Tulip breaking is one of the rare diseases in which the infected host is improved from a commercial and esthetic point of view. The chief symptom develops in the flowers: these, instead of being the usual solid color, become beautifully variegated, striped and mottled. This change in the flower's color is due to segregation of the anthocyanin pigment in the epidermis of the petals as fine featherings about the margin or in irregular stripes up the middle of each segment. Between these brightly colored streaks appear patches of more or less clear ground color, usually yellow or white. In addition to the flower symptoms, some tulip varieties show striping or mottling of the leaves.

Tulip breaking has been quite generally considered, in recent times at least, to be caused by a virus, and it has long been known to be readily transmissible by inoculation. Certain aspects of the disease, however, suggested to McWhorter that it was not a simple infection. For instance, in natural spread tests great variation in the types of breaks occurring were noted: the flowers of some infected plants were extremely dark, while those of other infected plants were very light. These two types of disease manifestation tended to be maintained in subsequent serial passage to normal tulips. Then, too, in some typically

"broken" clumps of tulips, distinctly darker or lighter flowers might appear at the margins of the clumps. This observation suggested to McWhorter that the light and dark "breaks" might be accounted for by two viruses which move at unequal rates through clumps and hence induce different symptomatic expressions in different shoots of the same clump. He therefore advanced the theory that tulip breaking, as it occurs naturally, results from the action of two viruses.²⁰ One of these viruses carries a color-adding factor and produces no visible effect on leaves; the other removes flower color and strongly stripes the leaves.

In subsequent work McWhorter proved the correctness of his theory by segregating each of the viruses, by studying their separate activities in tulips and by demonstrating that known mixtures of the two produced typical breaks.²¹ The two viruses in pure form cause singularly distinct symptoms. The color-removing virus (tulip virus I) inhibits chlorophyll formation, greatly restricts growth and is directly responsible for the recognition of tulip breaking as a disease. It is an extremely lethal virus, and in McWhorter's experience all plants infected with it in pure form died out during the season in which they exhibited symptoms. The color-adding virus (tulip virus II), on the other hand, has no effect on the ground tissue of the flower or on the ground color, stimulates epidermal pigmentation, has no visible effect on the leaves and has little effect on growth. It is not a lethal virus. Mixtures of the two viruses in the proper proportions reproduce faithfully the true picture of tulip breaking. The correct proportions are, however, definite within quite narrow limits. If the proportions found necessary by McWhorter are true criteria, a typical break may be considered an expression of viruses I and II in which the concentration of the color-adding II is at least ten times that of the color-removing I. Mixtures in these proportions appear to be physiologically balanced, and virus II prevents the lethal and growth-inhibiting effect of virus I. Because of this inherent antagonism between the two naturally associated viruses, McWhorter has applied the term "antithetic" to describe their mutual relationship in the production of tulip breaking. Tulip breaking thus differs from the other complex infections already discussed, in which the end results were achieved by additive effects of the two agents.

INFECTIOUS MYXOMATOSIS OF RABBITS

Infectious myxomatosis may be defined as an acute, highly infectious, very fatal disease of rabbits caused by *Virus myxomatosum*. Its salient observable features are hyperemic, edematous, myxomatous swellings at the site of inoculation followed by similar swellings at all

20. McWhorter, F. P.: *Phytopathology* **22**:998, 1932.

21. McWhorter, F. P.: *Phytopathology* **25**:254, 1938.

mucocutaneous junctions. Death ensues as a rule in from eight to twelve days after inoculation. Recoveries are almost unknown.

Many will doubtless consider that infectious myxomatosis has little claim to inclusion in a discussion of complex infections. However, the disease bears certain similarities to known members of that group, and I am including it in order to point out these resemblances.

Several years ago infectious fibroma was observed occurring naturally in wild cottontail rabbits. The causative virus proved readily transmissible to laboratory rabbits and in them produced a local swelling at the site of inoculation, comprised largely of young connective tissue cells and resembling the growths occurring naturally in the cottontail rabbits.²² The disease was named infectious fibroma, and the causative agent became known as the fibroma virus. In domestic as well as in cottontail rabbits the disease was entirely benign. There were no general signs of illness; the sole effect of the virus was the production of a fibromatous swelling at the site of inoculation; and the disease was not contagious. In all of these respects infectious fibroma differed markedly from infectious myxomatosis, which was highly fatal, highly contagious and characterized by occurrence of metastatic lesions at all mucocutaneous junctions. The fibroma did, however, bear a superficial resemblance to the local lesions produced by myxoma virus in that young connective tissue cells formed the bulk of both growths. Because of this superficial similarity, the possibility of an immunologic relationship between the causative agents of the two diseases was considered.

Domestic rabbits that had recovered from fibroma were inoculated with myxoma virus. In some of these only a localized myxomatous swelling developed at the site of inoculation, and there were none of the general symptoms of myxomatosis. In others a disease developed that clinically looked like true myxomatosis but differed in that the animals recovered. A very few of the rabbits which had recovered from fibroma died of myxomatosis.²³ It was clear from such results that previous infection with fibroma virus usually made rabbits quite highly resistant to myxoma virus. That this resistance was not of the same category as the cross immunity produced by two identical viruses for one another was indicated by the fact that rabbits which had recovered from fibroma almost always acquired lesions and signs of myxomatosis; they were incapable of destroying injected myxoma virus, and their serum did not neutralize myxoma virus sufficiently to prevent death, though Berry and Lichty²⁴ subsequently showed that the serum

22. Shope, R. E.: *J. Exper. Med.* **56**:793, 1932.

23. Shope, R. E.: *J. Exper. Med.* **56**:803, 1932.

24. Berry, G. P., and Lichty, J. A., Jr.: *J. Bact.* **31**:49, 1936.

of such rabbits does prevent the appearance of local myxomatous lesions. The relationship in the reverse direction was that of a true cross immunity, for rabbits which had recovered from myxoma were solidly immune to fibroma; they inactivated injected fibroma virus, and their serum neutralized fibroma virus. The conclusion was drawn that, though the fibroma and myxoma viruses were immunologically related, they were not identical viruses and fibroma virus did not constitute merely a mild strain of the myxoma agent.²⁵

Rather it seemed that the differences could be explained best on the basis of partial duplication of the antigenic components comprising the two viruses, the myxoma virus containing all the components found in the fibroma virus but the fibroma virus being antigenically only a partial replica of the myxoma virus. In line with this explanation the possibility was considered that the agent so long known as *Virus myxomatosum* might be, as Rivers²⁵ had, on other grounds, previously suggested, actually composed of more than one virus. The immunologic relationships between the fibroma and myxoma viruses were in accord with the possibility that *Virus myxomatosum* might be composed of fibroma virus and some other perhaps hitherto unknown virus. Such an explanation would adequately explain why *Virus myxomatosum* immunized completely against fibroma virus, one of its components, while the fibroma virus, being but a part of *Virus myxomatosum*, gave correspondingly only partial immunization.

In an effort to demonstrate this hypothetic complexity in *Virus myxomatosum*, serial passage of the virus through hosts that conceivably might favor survival of one or the other component was conducted. It was reasoned that, since fibroma virus had come originally from wild cottontail rabbits and was apparently a natural pathogen for this species, it might be segregated and obtained pure by the serial passage of *Virus myxomatosum* through cottontail rabbits. In like manner, it seemed possible that serial passage of *Virus myxomatosum* through fibroma-immune rabbits might result in the survival of only the hypothetic second virus. However, both sets of experiments yielded only unaltered *Virus myxomatosum*,²⁶ and no experimental evidence was obtained to support the suspicion that *Virus myxomatosum* might be a virus complex.

But Berry and Dedrick²⁷ were more successful in demonstrating the complexity of *Virus myxomatosum*, though they visualized this complexity in a light quite other than that outlined in the preceding paragraph. They considered the possibility that the fibroma and

25. Rivers, T. M.: *Proc. Soc. Exper. Biol. & Med.* **24**:435, 1926-1927.

26. Shope, R. E.: *J. Exper. Med.* **63**:33 and 43, 1936.

27. Berry, G. P., and Dedrick, H. M.: *J. Bact.* **31**:50, 1936.

myxoma viruses might be but different strains of one basic virus and, if this were the case, might be amenable to transformation in a manner similar to that employed by Griffith²⁸ with pneumococcic types.

The method they employed consisted in inoculating domestic rabbits with a mixture of active fibroma virus and heat-inactivated myxoma virus. In animals inoculated with such mixtures myxomatosis developed that was typical in all respects. This result, startling as it seems, can be duplicated readily, and neither Hurst²⁹ nor I have experienced any difficulty in repeating the experiments. In performing a transformation experiment the myxoma virus may be heated in sealed tubes for thirty minutes at any temperature between 60 and 75 C. Even the lowest temperature used is several degrees above that required to destroy the infectivity of myxoma virus. The heat-inactivated myxoma virus is then mixed with a small amount of active fibroma virus before injection into test rabbits. Typical myxoma virus, readily transmissible in series and with all its killing propensities intact, is recoverable from the inoculated rabbits. The disease that this transformed virus causes bears no more resemblance to infectious fibroma than did the original myxomatosis from which the virus for heating was derived.

The experiments of Berry and Dedrick have been extremely painstakingly controlled, and it seems impossible that the result is explainable on the basis of survival of traces of active myxoma virus in their heated material. Furthermore, it appears unlikely that the thermostabile component of myxoma virus is itself a virus as one usually thinks of such an agent, because alone it produces no signs of infection in rabbits, it confers not even partial immunity to myxomatosis, and it does not, of itself, multiply in the rabbit. Berry and Dedrick have not yet expressed their own views as to the nature of the transforming agent, though in a preliminary publication they have suggested that it may lend virulence to fibroma virus in a manner analogous to bacterial haptens. Whatever its nature, however, it seems established that the Berry-Dedrick factor, in conjunction with the benign fibroma virus, causes a disease that is unsurpassed among infectious maladies for pathogenicity and deadliness. The factor may yet prove to be viral in nature, peculiar only with regard to its high thermal inactivation point and its requirement of the concomitant presence of fibroma virus for multiplication within the rabbit host; and it does multiply, because, once established, it is indefinitely transmissible in series.

This completes my consideration of the complex infections, and in concluding I should like to emphasize two of the features of their causative agents that were not stressed earlier. The first concerns the

28. Griffith, F.: *J. Hyg.* **27**:113, 1928.

29. Hurst, E. W.: *Brit. J. Exper. Path.* **18**:23, 1937.

frequent extreme mildness of one or both of the agents when acting individually. In rugose mosaic of potato, for instance, an infection of potatoes with either the mottle or the vein-banding virus alone would attract little or no attention; and in swine influenza the bacterial component alone causes no recognizable illness, while the virus alone produces only an extremely mild disease. The combined effect, in all cases except tulip breaking, far exceeds that to be expected from the known activities of the agents singly. The second feature to be stressed concerns the occasional high pathogenicity of one of the components of an etiologic complex for another host. In tomato streak, for instance, the tobacco mosaic virus component alone may cause serious disease in tobacco; while in swine influenza the virus component alone will produce serious and sometimes fatal pneumonia in ferrets and regularly fatal pneumonia in white mice. If one wished to expand on the possible implications of these two features of the agents involved in the complex infections, one could readily speculate that they might have an important bearing with regard to the future appearance of new diseases of either simple or complex etiologic background. For instance, there is no way of knowing what a congregation of latent and, alone, impotent agents may be lurking about, awaiting only the addition of another mild agent to cause serious disease. Neither can it be foretold when one of the mild agents of a complex will forsake its partner and take up residence alone in a host for which it is highly pathogenic. But these possibilities are purely speculative.

Another fact of considerable interest that was not emphasized is that in the case of each one of the seven diseases discussed a simple "one agent" causation was in the beginning considered probable or proved. Some minor discrepancy or fortuitous observation led eventually to the discovery that two agents were involved. This seems to indicate that those who are studying infectious diseases are thinking largely in terms of one instead of two causal agents. They are still under the influence of the spirit of Koch's postulates and find it difficult to abandon even occasionally the concept that for each infectious disease there must be a single specific etiologic agent. If there is anything at all to be learned from present knowledge of the complex infections, it is that an infectious agent must fully explain and account for all of the features of a disease with which it is associated before it is accepted as the sole cause of that disease. Investigators must think more often in terms of two factors if they are to gain full understanding of all the infectious diseases.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, Etc.—Marcos Fernan-Nunez, professor of pathology and bacteriology in Marquette University, Milwaukee, is now chairman of the cancer committee of the State Medical Society of Wisconsin.

Julius M. Rogoff, visiting professor of physiology in the University of Chicago, has been appointed professor of endocrinology in the University of Pittsburgh.

C. H. Andrewes, pathologist, National Institute for Medical Research, and H. M. Turnbull, professor of morbid anatomy, London Hospital, have been elected fellows of the Royal Society.

William H. Park, director of the laboratories of the New York City Health Department from 1894 until his retirement in 1936, has died, aged 76 years.

William C. Thro, professor of clinical pathology at Cornell University Medical College from 1918 to 1937, has died at the age of 64 years.

Maurice N. Richter, since 1928 assistant professor of pathology in the College of Physicians and Surgeons of Columbia University, has been promoted to a professorship and appointed executive officer of the department of pathology in the New York Post-Graduate Medical School and Hospital.

Robert A. Moore, assistant professor of pathology at the Cornell University Medical College, New York, has been appointed professor of pathology at Washington University, St. Louis.

Alfred Stengel, vice president in charge of medical affairs of the University of Pennsylvania and emeritus professor of medicine, died on April 10, 1939, at the age of 70. Dr. Stengel was a member of the editorial board of the *ARCHIVES OF PATHOLOGY* from the beginning.

CORRECTION

In the article by Dr. L. A. Emge entitled "Sarcomatous Degeneration of Transplantable Mammary Adenofibroma of the White Rat," which appeared in the July 1938 issue (*ARCH. PATH.* 26:429, 1938), the letters were inserted in figure 2 in horizontal instead of vertical order, but the legends were not changed accordingly. To make the parts of the illustration correspond with the legends, the following changes are needed: C should be B, E should be C, B should be D, and D should be E.

Abstracts from Current Literature

TO SAVE SPACE THE ORIGINAL TITLES OF ABSTRACTED ARTICLES SOMETIMES ARE SHORTENED

Experimental Pathology and Pathologic Physiology

DISTURBANCES OF THE BLOOD AND LYMPH CIRCULATION IN THE ABDOMEN. W. D. GATCH, Surg., Gynec. & Obst. 66:322, 1938.

The problem of whether the intra-abdominal circulation is impeded by greatly increased intra-abdominal pressure was solved experimentally by determining the systolic blood pressure, the intra-abdominal pressure and the tension in the vena cava and then elevating the intra-abdominal pressure by injecting physiologic solution of sodium chloride into the peritoneal cavity. As the pressure within the abdominal cavity increased, the pressure in the vena cava kept pace with it so that the two were always equal. When the intra-abdominal pressure equaled the systolic blood pressure, all flow through the abdominal organs ceased, and they became white and bloodless. Thus it seems that the heart forces enough blood through the capillaries to maintain an intravenous pressure equal to that within the abdomen and further that the intra-abdominal circulation cannot be stopped by any increase of intra-abdominal pressure lower than the diastolic blood pressure. When the pressure on a capillary equals the diastolic blood pressure, the flow of blood through the capillary occurs only in systole and consists of a series of brief spurts. The resulting volume of the blood flow is insufficient to maintain the normal activities of the tissues. Under the conditions of the experiment the absorption of salt solution is slow.

The effect of bowel distention and that of venous obstruction limited to a single abdominal organ were likewise studied. It was demonstrated that the venous outflow from a distended bowel decreases as the intrainestinal pressure increases and ceases when the intrainestinal pressure equals the systolic blood pressure; that distention of the bowel causes no venous congestion thereof; that the bowel is not injured by intrainestinal tension short of that necessary to rupture it, provided the pressure is applied for a short time only; that after deflation the bowel can be made to contract normally and displays no microscopic evidence of damage; that the function of the bowel as shown by its power of absorption is maintained fairly normal in the presence of intrainestinal pressure lower than the diastolic blood pressure but ceases when the intrainestinal pressure is higher than this; that distention of the bowel causes no edema of its wall but diminishes or abolishes secretion by its mucosa; that loops of bowel kept tightly distended for a number of hours gradually increase in diameter and may remain viable if the pressure within them does not increase as fast as their walls are stretched, enlargement under these conditions diminishing the pressure on the capillaries of the bowel wall and permitting a resumption of the blood flow. The effects of distention on the appendix and on the cystic ovary are much the same as on the intestine. The gallbladder, however, behaves differently. After ligation of the cystic duct the gallbladder contracts within a few hours, which is due to rapid absorption of water from its contents; ligation of the common duct alone brings about great distention of the gallbladder, for the power of removing water from its contents is insufficient to cope with the constant arrival of more fluid by way of the cystic duct.

In the presence of venous obstruction manometer readings indicate a rise of pressure equal to that of the systolic blood tension, proving that in venous obstruc-

tion the systolic blood pressure is transmitted through the capillaries into the veins. Under these circumstances the capillaries rupture, and there follows a massive extravasation of blood into the tissue spaces.

Disturbances of the blood flow are always accompanied by alterations of the lymph flow: An elevation of intrainestinal pressure beyond a certain level abolishes the flow of lymph in the abdominal wall; a slight rise in intravenous pressure increases the flow of lymph, while complete venous obstruction abolishes it.

WARREN C. HUNTER.

ALLERGIC HYPERERGIC INFLAMMATION EVOKED BY AUTOSERUM. W. EICKHOFF, *Virchows Arch. f. path. Anat.* **301**:264, 1938.

Attempts to sensitize an animal to its own blood or serum have led to conflicting results because, according to Eickhoff, there could be no certainty that the blood or serum used for reinjection had the same chemical composition as that used for the first injection. Eickhoff overcame this objection by withdrawing from rabbits and guinea pigs by cardiac puncture a quantity of blood that would yield sufficient serum for a first, or sensitizing, injection and a second, or provocative, one. The two portions of serum were inspissated in vacuo at the same time by the method of Flosdorf and Mudd. Sealed under vacuum, the material was kept on ice until needed. Then sufficient distilled water was added to restore the original volume of the serum. Solutions so prepared were clear and colorless. The first injection was made three weeks after the cardiac puncture, and the second three weeks after the first, both being made subcutaneously and at the same site. The first injection had no detectable local effect. The second injection of the autoserum led to local swelling of the skin. Histologic examination of such an area forty-eight hours after the injection revealed edema of the subcutis, separation of its fibrous elements and marked cellular infiltration, the picture being identical with that of Arthus' phenomenon. The infiltrating cells were leukocytes, eosinophils and histiocytes. Tissues examined at later periods showed fibroblastic proliferation. If the second dose of autoserum was given by intravenous or intracardiac injection it produced anaphylactic shock.

O. T. SCHULTZ.

Pathologic Anatomy

STENOSIS OF THE SPLENIC VEIN IN CHILDHOOD. J. HÖRA, *Virchows Arch. f. path. Anat.* **300**:670, 1937.

Thrombosis of the splenic vein in the adult leads to a characteristic triad of splenomegaly, gastric hemorrhage and secondary anemia, which permits clinical recognition of the condition. In children a somewhat similar complex of symptoms occurs, but operation or necropsy fails to reveal thrombosis or obstruction of the splenic vein. The noncommittal clinical term "stenosis of the splenic vein" has been applied to the condition in children. Failure to detect obstruction of the splenic vein has led to the supposition that the obstruction to the splenic circulation lies within the spleen itself. In children splenectomy usually leads to recovery; hence there are few records of observations made at necropsy. Höra presents a detailed histologic study of 2 spleens removed surgically, the first from a boy aged 8 years and the second from a girl aged 9 years. Neither grossly nor microscopically was there evidence of disease or of obstruction of veins outside the spleen. In each instance the spleen was enlarged to about four times the normal size. The splenomegaly was due to hyperplasia of the red pulp, with marked prominence of the sinusoids. Congestive hemorrhages had occurred in the trabeculae, and siderofibrous nodules had been formed, but there were no alterations within the spleen itself that would account for interference with the

circulation through the spleen. The cause must be sought outside the spleen. In the absence of anatomic changes in the splenic vein one must consider functional stasis of the vein or obstruction of the portal circulation.

O. T. SCHULTZ.

SPECIFIC CELLULAR CHANGES DUE TO ELECTRICITY. S. JELLINEK, *Virchows Arch. f. path. Anat.* **301:28**, 1938.

As characteristic and specific effects of the passage of electricity through the body, the director of the Institute for Electropathology of the University of Vienna describes two types of cellular change. These occur in and immediately adjacent to electric marks that reveal no evidence of burning. The first is a spiral deformation of the nuclei of the media of blood vessels, as if the nuclei had been subjected to a twisting force in opposite directions applied to their poles. Such deformed nuclei were observed in the tissue adjacent to scars of lesions caused by electric current and sustained, respectively, three and five years previous to death, an observation which the author characterizes as remarkable. The other type of change was noted especially in the epithelium of the hair follicles. The nuclei were elongated, needle shaped, and had a wavelike geometric arrangement, which the author likens to the lines of force in an electric field. Both types of change were observed in rabbit tissues experimentally excised by the high frequency electric surgical knife, at a slight distance from the surface in contact with the knife. These observations, the author suggests, may be indicative of possible danger in the use of the high frequency knife or of the diathermy current.

O. T. SCHULTZ.

RELATION OF ANOMALIES OF THE CIRCLE OF WILLIS TO ANEURYSM OF THE BASE OF THE BRAIN. A. SLANY, *Virchows Arch. f. path. Anat.* **301:62**, 1938.

Brief summaries are presented of 26 cases of aneurysm of the circle of Willis that led to fatal intracranial hemorrhage. The subjects had come to necropsy in Priesel's institute, Vienna, during the preceding ten years. In 14 cases the aneurysm was associated with an anomaly of the vascular circle. The frequency of the association suggests that there may be a causal relationship between anomaly and aneurysm of the circle of Willis.

O. T. SCHULTZ.

THE PYELONEPHRITIC CONTRACTED KIDNEY. T. FAHR, *Virchows Arch. f. path. Anat.* **301:140**, 1938.

In a 46 page article, in which the material is excellently organized and set forth in language easy to read, Fahr presents a study of the pathologic aspects and a discussion of the genesis of the pyelonephritic contracted kidney, based on 80 cases. He traces the process from its inception as subacute pyelitis, which may involve the entire pelvis or only one or more calices, through stages of ascending proliferative interstitial inflammation of the medulla, to a final stage of almost complete replacement of the kidney by fibrous tissue, with contraction. He agrees in general with Staemmler but does not agree that in every instance the process passes through a stage of cystic dilatation of the tubules or a stage of struma-like transformation of the kidney. He thinks it is necessary to recognize two different types of ascending inflammatory process. In one of the ascending interstitial inflammation is more diffuse, spreads more rapidly upward into the kidney, with fibrous replacement of tubules and glomeruli but without struma-like transformation of the organ. In the other type the process ascends more slowly and is associated with fibrosis of the medulla; with this change the cystic stage described by Staemmler develops. But such cystic transformation is not always the result of the ascending inflammation. It may have existed previous

to the development of the latter in hypogenetic areas of the kidney or in an organ which is diffusely hypoplastic. Such hypogenetic areas seem to be more prone to ascending inflammation than previously normal kidneys. Hypogenetic renal tissue is also more prone to malignant nephrosclerosis and chronic glomerulonephritis. Fahr suggests that the interstitial nephritis associated with dwarfism or rickets is ascending pyelonephritis in a congenitally hypogenetic kidney. He believes it is necessary to recognize a form of nephritis which he terms hypogenetic nephritis. He considers chronic ascending nephritis the most frequent form of renal inflammation.

O. T. SCHULTZ.

WIDENING OF THE CRANIAL SUTURES. A. E. SITSEN, *Virchows Arch. f. path. Anat.* **301**:287, 1938.

Sitsen gives a detailed description of the pathologic alterations of the bone at the suture margins in 5 cases of widening of the cranial sutures. The series includes 1 case of spongioblastoma of the brain in a child, 1 case of sympathicoblastoma in a child with metastasis to the skull and 3 instances of suppurative osteomyelitis of the cranial bones. Widening of the sutures may be brought about by abnormal mobility of the irregular bone margins or by separation of these bone margins. Increased mobility may be due to (1) resorption of bone and its return to the fetal state, as brought about by increased intracranial pressure, (2) to destruction of bone by a metastatic tumor situated near a suture and (3) to destruction of bone by suppurative inflammation. Actual separation of the suture margins requires an increase in intracranial pressure and can occur only in early life, when the sutures have not yet united, or in later life, when the bony margins of the sutures are altered or destroyed.

O. T. SCHULTZ.

CARCINOGENETIC ACTIVITY, STRUCTURE AND CHEMICAL REACTIVITY OF POLYNUCLEAR AROMATIC HYDROCARBONS. L. F. FELSER, *Am. J. Cancer* **34**:37, 1938.

A survey and analysis are made of the chemical and biologic investigations of hydrocarbon carcinogenesis, with significant conclusions bearing on further work in this field.

VARIATION IN THE CREATINE CONTENT OF HUMAN MUSCLE AT AUTOPSY. C. R. LINEGAR, T. T. FROST and V. C. MYERS, *Arch. Int. Med.* **61**:430, 1938.

The creatine content of the heart is low at birth but progressively increases until within a few months it is equal to that found in adults. The saturation level for the creatine of cardiac muscle is reached much earlier than that for the creatine of voluntary muscle. In cardiac decompensation the creatine content of the heart, i. e., of both the left and the right ventricle, is definitely lowered in comparison with average values. It is also usually slightly lowered in diabetes and carcinoma. On the other hand, the creatine content of the muscles of the left and right ventricles may be considerably increased in uremia uncomplicated with heart failure and in pneumonia in some cases. The creatine content of voluntary muscle (the pectoralis major muscle being taken as an example) is reduced in diabetes and carcinoma and increased in uremia uncomplicated with heart failure and in the pneumonias, compared with average values. The creatine content of cardiac and voluntary muscle may be reduced or increased in fairly constant ratios, but the major evidence points to the conclusion that variations in these two distinctly different muscles are not related except when the creatine contents of both voluntary and cardiac muscle are elevated, probably as a result of retention of nitrogen.

FROM AUTHORS' SUMMARY.

Pathologic Chemistry and Physics

MINERALS IN NORMAL AND IN PATHOLOGIC BRAIN TISSUE, STUDIED BY MICRO-INCINERATION AND SPECTROSCOPY. L. ALEXANDER and A. MYERSON, Arch. Neurol. & Psychiat. **39**:131, 1938.

The microincineration method enables one to determine the relative distribution of ash in various parts of normal and of pathologic nerve tissue. The gray matter is rich in ash; the white matter is poor in ash. The mineral distribution in the various parts of the neuron varies. For instance, in the ganglion cell the nucleus and Nissl bodies contain rich deposits of ash, while the intracellular neurofibrils contain little or no mineral ash; the lipid of the myelin is free from mineral ash, and the axons contain small amounts. With hemorrhages, inflammation, tuberculous sclerosis and tumors there is hypermineralization; in ischemic necrosis, in plaques of multiple sclerosis, in areas of cortical atrophy and in cell disease of patients with amaurotic family idiocy there is demineralization.

Spectroscopic studies revealed that the white matter contains about twice as much phosphorus as the gray matter, while the gray matter is richer in sodium, calcium and magnesium. The dried substance of the gray matter is richer in iron, but potassium, manganese and copper are about evenly distributed. By the spectroscopic method the relative mineral content was studied in the gray and white substances of the brains of newborn infants and in those of normal adults, also brains showing one or the other of the following conditions: softening of the brain, multiple sclerosis, dementia paralytica, encephalitis due to poisoning with lead, cerebral edema and tumors. In multiple sclerosis the plaques showed twice as much iron as normal white matter of the same brain; calcium was decreased in the plaques, and phosphorus showed no significant changes. The reason for the discrepancy between the findings by the spectroscopic and the microincineration methods of studying multiple sclerosis was that in multiple sclerosis the scavenger glia cells appropriated, as it were, most of the minerals and thus depleted the tissues. In dementia paralytica no absolute increase of iron was found, while in lead encephalitis more lead was deposited in the gray than in the white substance.

GEORGE B. HASSIN.

SERUM ENZYMES AND FERMENTATION TESTS. N. E. GOLDSWORTHY, J. E. STILL and J. A. DUMARESQ, J. Path. & Bact. **46**:253, 1938.

Horse serum contains amylase and maltase even after storage for many weeks at 4 C. These enzymes can lead to a false interpretation when the serum is added to a fermentation medium. To avoid this difficulty, the serum should be heated for sixty minutes at 65 C. before being added to the medium. There is disagreement between the results of some authors who have investigated the enzyme content of serum in various animal species. Certain factors other than enzymes which may influence the apparent result of a fermentation test are discussed.

FROM AUTHORS' SUMMARY.

CENTRIFUGATION OF THE ELEMENTARY BODIES OF INFECTIOUS MYXOMATOSIS OF THE RABBIT. C. E. VAN ROOYEN and A. J. RHODES, Zentralbl. f. Bakt. (Abt. 1) **140**:117, 1937.

A speed of 15,000 revolutions per minute for two hours caused deposition of the elementary bodies. The supernatant fluid was noninfective, but the centrifuged sediment containing the elementary bodies, on intradermal injection into rabbits produced typical myxomatous papules.

PAUL R. CANNON.

Microbiology and Parasitology

SUPERINFECTION IN MALARIA. L. T. COGGESHALL and H. W. KUMM, *J. Exper. Med.* **68**:17, 1938.

Protection tests were utilized to determine the effect of superinfection on the potency of immune serum from monkeys chronically infected with *Plasmodium knowlesi*. The results of these tests showed that: 1. In two groups of monkeys with comparable infections the immune serum of 8 monkeys which had been superinfected on seven separate occasions over a period of two months was much more potent than that of a group of 7 monkeys in which the chronic course of infection was allowed to continue without the introduction of a superinfection. 2. After a series of nine more intense superinfections the serum from the same two groups of monkeys contained no demonstrable protective antibodies. 3. The serum from 8 of the 10 monkeys in the original two groups showed a relatively high concentration of protective antibodies following a month's rest and a single superinfection. 4. The results of the experiments indicate that it is possible to increase the potency of immune serum by superinfections but that it is also possible to obtain a decrease in the protective property of the serum by too severe superinfections.

FROM AUTHORS' SUMMARY.

CUTANEOUS INFECTIVITY IN POLIOMYELITIS. W. J. GERMAN and J. D. TRASK, *J. Exper. Med.* **68**:125, 1938.

Bilateral olfactory neurectomy did not prevent experimental poliomyelitis following intravenous or intracutaneous inoculation of the virus. Various operative procedures increased the susceptibility of monkeys to this infection.

FROM AUTHORS' SUMMARY.

A POSSIBLE MECHANISM OF LOWERED RESISTANCE TO PNEUMONIA. W. J. NUNGESTER and R. G. KLEPSE, *J. Infect. Dis.* **63**:94, 1938.

Mucin injected intrabronchially favored the production of pneumonia in rats sprayed one day later with pneumococci. Certain factors, as exposure to cold, prolonged deep ether anesthesia or alcoholic intoxication, increased the aspiration of mucous material placed in the noses of white rats. Such factors also increased the incidence of pneumonia if pneumococci and mucin had been inoculated intranasally. Cold or alcoholic intoxication were found to interfere with the closing of the glottis, thereby permitting the aspiration of mucin and pneumococci.

FROM AUTHORS' SUMMARY.

TUBERCULOUS INFECTION FROM TALCUM USED TO DRY GLOVES USED FOR NECROPSIES. R. OEHNELL, *Zentralbl. f. allg. Path. u. path. Anat.* **69**:324, 1938.

Dissemination of tubercle bacilli by talcum powder used to dry gloves worn at necropsies is reported. Oehnell carried out his researches designated A and B, with observations as follows. At hospital A, from 8 to 9 bodies dead of pulmonary tuberculosis were examined each month. The attendants frequently washed their rubber gloves hurriedly with soap and water, then gave them a preliminary drying on a towel. Talcum sprinkled from a shaker was used to complete the drying and often was scattered in a cloud when imprisoned air was used to turn the gloves right side out. Guinea pigs placed within a meter of the tablet on which the excess talcum fell, developed widespread tuberculous lesions. Others exposed to talcum which had not come in contact with gloves remained free from disease when kept about 10 meters from the tablet.

In hospital B, the gloves were powdered in a large glass cylinder kept in an antechamber of the morgue. Talcum was used repeatedly until the container was empty. Only from 1 to 2 bodies dead of tuberculosis were examined in this institution per month. Guinea pigs kept close to the talcum container remained healthy and had no tuberculous conditions post mortem. The studies indicate a possible source of tuberculous and other infections in man exposed to talcum dust contaminated by bacteria adherent to gloves used in morgues.

GEORGE J. RUKSTINAT.

BRUCELLIASIS IN JAPAN AND MANCHUKUO. H. HIROKI, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* 92:382, 1938.

From 10 to 20 per cent of cattle in Japan were found infected with *Brucella abortus*. *Br. abortus* infection of man is not rare, but there are no known instances of infection of man with *Brucella melitensis*. *Brucella* infection is considerably greater in Manchukuo, in man as well as in cattle. The serum of about 10 per cent of the persons working on cattle and sheep farms gave positive agglutination or complement fixation with *Br. abortus* or *Br. melitensis*. Some of these had no clinical manifestations and no history of the disease.

I. DAVIDSOHN.

Immunology

BRUCELLA PRECIPITIN SYSTEMS. R. B. PENNELL and I. F. HUDDLESON, *J. Exper. Med.* 68:73 and 83, 1938.

It has been shown that the precipitation by the endoantigens of the three species of *Brucella* of their homologous antibodies may be described by equations developed from the law of mass action. The endoantigens may be used in calibrating accurately *brucella* antisera. The nitrogen-containing constituent of the endoantigens does not always seem to be intimately connected with the ability to precipitate the specific antibodies.

Quantitative cross precipitation studies with goat antisera show the three endoantigens of *Brucella* to be serologically distinguishable. Although the endoantigens of *Brucella abortus* and *Brucella suis* are very similar, they do not react identically, which permits the two organisms to be distinguished serologically. These differences in cross precipitation may be used to identify an organism of the *brucella* group or to determine the organism responsible for a *brucella* antiserum.

FROM AUTHORS' SUMMARIES.

IMMUNITY AFTER ENCEPHALITIS VIRUS VACCINATION. L. T. WEBSTER, *J. Exper. Med.* 68:111, 1938.

Susceptible mice that are given subcutaneous or intraperitoneal injections of 15,000 intracerebral lethal doses of St. Louis encephalitis virus acquire immunity in from four to seven days to from 1,000 to 1,000,000 lethal doses given either intracerebrally or intranasally. This immunity persists for from four to six weeks, then decreases gradually and after from eight to twelve weeks disappears. More than 1,000 intracerebral lethal doses of virus given as a vaccine does not materially increase the amount or duration of the immunity; less than 1,000 doses gives little or no immunity. Test virus injected intracerebrally into immunized mice induces few lesions and is rapidly destroyed; instilled intranasally, it rarely reaches the olfactory lobes or brain. While immunity is maximum, circulating neutralizing antibodies are not detectable. Moreover, the immunity is not affected by endothelial cell blockade or by splenectomy. A few moments after the immunizing virus is given, it can be recovered from the blood in relatively high concentration.

After twenty-four hours, the blood no longer contains demonstrable virus nor do any organs thus far tested except the spleen. The brain and cord remain entirely normal. The spleen, however, becomes enlarged and harbors virus for as long as thirty days.

FROM AUTHOR'S SUMMARY.

TOXIN PRODUCTION BY *BACILLUS HISTOLYTICUS*. L. E. WALBUM and G. E. REYMAN, J. Path. & Bact. **46**:315, 1938.

Under the experimental conditions described *Bacillus histolyticus* grows luxuriantly in ordinary broth containing 1 per cent peptone irrespective of the presence of dextrose. The production of toxin appears to be greatest in broth without addition of dextrose or in *Bacillus coli*-fermented broth. The peptone content of the medium has a considerable effect on the toxin production, the production increasing with increase in the concentration of peptone. The greatest production of toxin occurs in peptone broth containing pieces of meat. This medium gives a substantial and steadily increasing production of precisely those protein split products which are of such importance for the growth of the bacteria and for the production of toxin. *B. histolyticus* toxin has its maximal point of stability in the neighborhood of p_H 6. The optimal reaction for the albumose-digesting enzyme lies around p_H 7.

FROM AUTHORS' SUMMARY.

AGGLUTINOGENS RESEMBLING M AND N FACTORS IN MONKEYS. P. DAHR and H. LINDAU, Ztschr. f. Immunitätsforsch. u. exper. Therap. **92**:335, 1938.

One anti-M testing fluid clumped the red cells of some old world lower apes, while other anti-M serums failed to do it. The observation indicates that there are differences in the anti-M agglutinating serums. An explanation is offered for that difference: The M agglutinin is composed of fractions, some of which are present in certain animal species. Occasional rabbits may have one or the other of these fractions in their red cells or in other tissues, a circumstance influencing the structure of antisera produced by inoculating them with human red cells of group OM. The failure to find the M property in an animal species is of little significance if only one anti-M testing fluid has been used. The resemblance of the M agglutinin of monkeys to that of man increases with the zoologic proximity to man. The N agglutinin was found only rarely in monkeys. Here, again, the agglutination reaction, indicative of the presence of the N factor, was demonstrated only with some of the anti-N serums, showing that they, too, differ as do the anti-M serums.

I. DAVIDSOHN.

ELIMINATION OF THE B AGGLUTINOGEN IN SALIVA. F. KAUFERZ, Ztschr. f. Immunitätsforsch. u. exper. Therap. **92**:460, 1938.

The B agglutinin of man consists of at least three fractions, B_1 , B_2 and B_3 . B_1 has thus far been found only in man and in some anthropoid apes. B_2 and B_3 are regularly present in rabbits, cats and some other animals. B_3 is found in guinea pigs, some dogs, elephants and some lower apes. Human anti-B serums differ in composition: Most of them have β_2 and β_3 agglutinins, which are directed against red cells with the corresponding agglutinogenic fractions; some serums have in addition to the aforementioned agglutinins β_1 agglutinin; none are known with only the β_3 agglutinin. The B agglutinin which is eliminated in the saliva does not always consist of the same fractions that are present in the red cells of the same person; in 13 of 50 salivas of B persons only fraction B_1 was present; if these 13 persons had been tested with one of the common anti-B serums with only β_2 and β_3 agglutinins they would have been labeled noneliminators. Only

serum with the β_1 agglutinins in addition to the two other fractions can be used for a reliable determination of the elimination of B agglutinin. By proper absorption of human anti-B serums with rabbit red cells, containing the B_2 and B_3 fractions, and with the red cells of the guinea pig, containing the B_2 fraction, serums can be prepared which hold isolated agglutinins β_1 or β_2 .

I. DAVIDSOHN.

THE SHWARTZMAN PHENOMENON. G. ALBUS and K. FISCHER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **92**:472, 1938.

Rabbits were sensitized with an intraperitoneal injection of pollen extract. The presence of sensitization was established by means of the passive transfer of Prausnitz-Küstner. The sensitized animals were inoculated intracutaneously in one area with a filtrate of a culture of *Bacillus coli* and in another area with 0.3 cc. of a 1:10 dilution of the pollen extract. In both areas inflammatory reactions developed. Intravenous injections of 2 cc. of the filtrate of *B. coli* produced grossly and microscopically typical hemorrhagic lesions in 50 per cent of the animals. In no case did a hemorrhagic lesion develop only in one of the two prepared sites. In nonsensitized animals hemorrhagic lesions appeared only in the areas prepared with the bacterial filtrate. Normal rabbits were prepared locally by intracutaneous injections of *B. coli* filtrate. Intravenous injections of a mixture of pollen extract and serum from sensitized animals did not produce the phenomenon, but when animals were sensitized to the pollen and then given injections as described the phenomenon was produced in 25 per cent of them. The results indicate that the reaction that takes place between the pollen and the reagins is capable of preparing the skin for the Shwartzman phenomenon and further that the in vivo reaction between the injected pollen extract and the reagins in the sensitized animal can provoke the phenomenon in the prepared skin.

I. DAVIDSOHN.

Tumors

CLASMATOSIS IN THE MELANOBLAST. C. G. GRAND, *Am. J. Cancer* **33**:394, 1938.

The elimination of granules of melanin by the melanoblast occurs by active pinching off, or clasmatosis, of clumps of granules irregularly arranged along the length of its dendrites. Clasmatosis occurs either by fragmentation of the pseudopodium or by formation of a bud which breaks away from the side of the pseudopodium. The constancy of clasmatosis in the melanoblast and its occurrence in only one of the types of cell found in the tissue cultures of mouse melanoma argue against the phenomenon being accidental or due to an abnormal condition of the medium. The irregularity in the appearance of the granules in the macrophages is due to the ingestion by the macrophages of the fragmented debris of melanin previously eliminated by the melanoblasts. Clasmatosis, a physiologic mode of excretion first described by Ranvier, is at least one way in which the melanoblasts of the Harding and Passey mouse melanoma eliminate melanin.

FROM AUTHOR'S SUMMARY.

BASOPHIL ADENOMA OF THE PARS INTERMEDIA OF THE HYPOPHYSIS. A. T. RASMUSSEN and A. A. NELSON, *Am. J. Path.* **14**:297, 1938.

Two cases in which basophil adenoma originated from the pars intermedia are described. In the first case the only symptom referable to the hypophysis was high blood pressure. On account of the age of this patient (77 years) the association of the adenoma with the hypertension is questionable. In the second case a number of the major characteristics of pituitary basophilism (adiposity, striae

atrophicae, hirsuties, high blood pressure, florid face) were present. In neither case were there any hyaline changes in the basophilic cells. There was considerable diffuse invasion of the neural lobe by these cells in both cases. The greater bulk of the data, however, does not indicate that there is any direct significant correlation between this invasion and hypertension.

FROM AUTHORS' SUMMARY.

THE HISTOLOGY OF THE INFECTIOUS FIBROMA IN RABBITS. C. G. AHLSTROM, J. Path. & Bact. **46**:461, 1938.

The infectious fibroma of the rabbit shows both inflammatory and neoplastic features; the early stages are chiefly granuloma-like, whereas neoplastic features dominate the later stages. The cells originate not only from fibroblasts but also from perivascular histiocytes and endothelial cells, groups of young capillaries with hyperplastic endothelium serving as centers for the proliferation of the young tumor. The cells show characteristic basophilic cytoplasmic inclusions, increasing in amount and in size with the age of the tumor. The epithelium overlying the intracutaneous fibroma shows inclusions in the form of eosinophilic granules and sometimes also hyperplasia. Regression of the fibroma occurs through a combination of necrosis and resorption, of which the former seems to be primary.

FROM AUTHOR'S SUMMARY.

PROPHYLAXIS OF OCCUPATIONAL CANCER. O. TEUTSCHLAENDER, Med. Welt **11**: 1267 and 1341, 1937.

Teutschlaender is the author of the so-called allobiotic theory of cancer, according to which the carcinogenic agent after a sufficiently long exposure produces in the body a condition of cancer readiness. That condition, or allobiosis, is characterized by the tendency of the body to react to irritating agents with neoplasia instead of with the usual reaction. A table presents all known instances of occupational cancer. Teutschlaender attempts to apply the three factors—(1) the constitution (allobiosis), (2) the carcinogenic agent and (3) the term of exposure—to the prophylaxis of occupational cancer. The applicability is illustrated in several industries, with particular consideration of the laws in Germany.

I. DAVIDSOHN.

TUMORS OF THE THYMUS AND THEIR ASSOCIATION WITH MYASTHENIA. R. A. OBIDITSCH, Virchows Arch. f. path. Anat. **300**:317, 1937.

Nine cases of tumor of the thymus are described. In 4 of the cases the growth was benign and was termed lymphoepithelial; the predominating cell was the small lymphocyte. In 5 cases the tumor was malignant and of the squamous epithelial type; this type the author terms a malignant medulla cell tumor. Only the benign tumors were associated with myasthenia. The benign tumors, which retained the function of the thymus, secreted an excess of a thymic substance causing myasthenia. Whether it acted directly on the muscle or on the nervous system or on the metabolism has not been determined.

O. T. SCHULTZ.

VIRULENCE OF THE CELLS OF TRANSPLANTABLE TUMORS. A. SYMEONIDIS, Virchows Arch. f. path. Anat. **300**:429, 1937.

Inoculability in the case of a tumor is an expression of intrarelationship between the properties of the host and those of the tumor cell. Ehrlich, influenced by bacteriologic terminology, stated that the virulence of a transplantable tumor is composed of two factors: (1) its ability to overcome the host's protective mechanisms and establish itself; (2) its energy of growth after it has "taken." Inoculability can be measured by the percentage of "takes" in a series of animals of uniform con-

stitution. Unless "takes" in 100 per cent of cases can be assured, the variation in "takes" is too great to be of much value as a measure of virulence. In the work of Symeonidis with the Ehrlich transplantable mouse carcinoma and with the Ehrlich tumor originally termed sarcoma but which Symeonidis considers to be carcinoma of an anaplastic type, "takes" were obtained in 100 per cent of each series of inoculated animals. Inoculability is a fixed property. In the course of the work the so-called sarcoma became much more highly virulent as measured by rapidity of growth, appearance of metastases and duration of life. During this period "takes" were obtained with a dose of as few as 3,000 suspended cells. With 10,000 cells, "takes" were obtained uniformly. Such an increase in virulence must be due to increased resistance on the part of the tumor cells to the protective mechanisms of the host. Such an increase in virulence occurring in the course of a series of animal passages is due to segregation out of the tumor of cells more resistant or less susceptible to the antagonistic protective mechanisms of the host. In determining the degree of virulence of transplantable tumors it is necessary to determine the "absolute minimum" of living tumor cells that will result in a "take," and the "optimal minimum" of the smallest number of cells that will result in "takes" in 100 per cent of cases. Malignancy is not identical with virulence, as the author uses the latter term, and malignancy is not a measure of the virulence of transplantable tumors. Successful inoculation after implantation of organs that contain no visible tumor tissue is due to microscopic metastases or to cells of high virulence that have been carried into the organ.

O. T. SCHULTZ.

Medicolegal Pathology

DIAGNOSIS AND MEDICOLEGAL IMPLICATIONS OF ALCOHOLIC INTOXICATION. S. SELESONICK, J. A. M. A. **110**:775, 1938.

It is important to have definite criteria as a basis for the diagnosis of alcoholic intoxication in accidents involving persons who have imbibed alcoholic beverages. The chemical determination of alcohol in a body fluid offers a scientific means of establishing whether or not a person has imbibed alcohol and of estimating the degree of alcoholic intoxication. Blood as a medium for analysis is preferable to spinal fluid, urine, saliva or expired air for the following reasons: It contains a negligibly small amount of nonalcoholic oxidizable material. Its alcoholic content represents the degree of alcohol saturation at the moment the blood sample is obtained. It is always available, and its extraction does not necessitate the active participation of the subject.

There are sufficient scientific data to prove that subclinical intoxication—or alcoholic intoxication in the biologic sense without any gross manifestations of drunkenness—can produce sufficient interference with psychomotor activity and neuromuscular coordination to render the affected person a public menace. The technic of determining the alcohol in the blood detects degrees of alcoholic intoxication which ordinarily escape detection by competent physicians. Criteria, therefore, must be established which include body fluid alcohol determinations as part of the diagnostic armamentarium.

FROM AUTHOR'S SUMMARY.

ASPIRATION OF AMNIOTIC LIQUOR. J. CAMERER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **29**:333, 1938.

Reports of the presence of amniotic liquor in the lungs and bronchi of newborn children are exceedingly variable. After the publications of Haberda, the microscopic demonstration of even sparse elements from the amniotic fluid in the lung was accepted as the cause of natural death. Camerer has examined the lungs of 212 infants, in 45 of whom autopsy revealed evidence of aspiration of amniotic fluid. In 28 others the evidence was questionable, and in the remaining 139 no such evidence was found. Of these children, 93 were stillborn, 48 died during

the first day, 47 lived a day, and 24 died during the first week. In the majority of the cases, tissues from one lung only were prepared, since the author was unable to find any difference in the content of the various lobes. There was, however, a great variation in the localization of the material within a lobe. At times, the aspirated masses were located in the large and small bronchi. At other times, these structures were empty, and the alveoli were distended with foreign material. Of the 212 bodies examined, only 3 did not contain any demonstrable amniotic component, and in these 3 there was lymphocytic and leukocytic infiltration partly in the alveoli and partly in the interstitial tissue. In the 67 lungs in which only sparse epithelial cells were found and no fat, the cells were scattered in the alveolar spaces but were never found in the bronchi. These findings seemed unassociated with pulmonary function, as they were noted in lungs which had breathed, in those which had not breathed and in those which were macerated. It also made no difference if the child was immature or post-mature, as their lengths varied from 32 to 56 cm. Because of the great constancy of these findings, the conclusion seemed justified that the process was physiologic.

In the lungs of 79 children, fat and epithelium were demonstrated in small or moderate amounts. It is remarkable that this aspiration has no after effects. In none of the author's cases was there an inflammatory reaction. It is evident, therefore, that these substances do not call forth a foreign body reaction or pneumonia. Large fat-laden wandering cells occurred in the alveoli involved. The rest of the fat was found in the endothelium of the alveolar capillaries in the form of fine droplets, especially in the older children. It appears, therefore, that the fat is taken up by the reticuloendothelial system of the lung and is there either changed or disposed of. The epithelial cells are preserved longer than the fat and are still stainable in macerated fetuses and in the lungs of children who have lived for eight days.

GEORGE J. RUKSTINAT.

Society Transactions

NEW YORK PATHOLOGICAL SOCIETY

MAURICE N. RICHTER, *Vice President, Presiding*

Regular Meeting, Feb. 23, 1939

ROBERT A. MOORE, *Secretary*

GLOMUS TUMOR OF THE ARM. ANDREA SACCONI and JOSEPH MENDELLOFF.

To date 106 cases of glomus tumor have been recorded in the literature. A case of glomus tumor in a 42 year old white man is described. The tumor occurred at the site of a "blue birthmark" after injury to that site. The "birthmark" was of ten years' duration, and the glomus tumor was of eight months' duration. The tumor cells appeared to be of myoblastic origin. The suggestion is made that there may have been a connection of these cells to the periglomerular nerves by means of nonmyelinated fibers. In view of the fact that the exact nature of the previous "blue birthmark" is unknown, it is difficult to postulate a relationship of the glomus tumor to it, although some relationship is strongly suggested.

DISCUSSION

AMOUR F. LIBER: This case is of interest not only because of the situation, outside of the tips of the digits, which is somewhat unusual, but because of an unusual clinical feature, the fact that the pain was referred to the region of the sixth rib, which belongs to a dermatome entirely different from that of any part of the upper extremity. In most cases of glomus tumor the pain is referred very often to the same nerve or at least to the same dermatome. This opens a number of curious vistas, that the pain may be referred to a region of the skin innervated not by the same spinal metamere but possibly by some common sympathetic innervation. I have had occasion to observe a case of glomus tumor in the wrist which in some ways is analogous to this one (lantern slide shown). I think the lantern slide is sufficient to demonstrate that one is dealing with a glomus tumor. The marker represents a length of 100 microns. The pain in this case had lasted ten years but was slight. Here again is a history of trauma, apparently slight, but sufficient to cause an ecchymosis in the painful region, and following this the pain was aggravated, and a visible growth appeared.

There is one other point. Very often small painful bluish tumors which have the same subjective features as glomus tumors are found in the extremities and on the body, particularly on the thighs and legs, and are frequently diagnosed clinically as glomus tumors. Often they are painful subcutaneous leiomyomas, to which Dr. Stout has called attention in his study, and of course they are easy to diagnose histologically. Clinically they are almost invariably called glomus tumors.

ANDREA SACCONI: I should like to add that the glomus tumor is a type of angioma, a complex type, in which the neuromyoblastic elements play an important role in the clinical manifestations and in the histologic appearances.

EFFECTS OF INTRAVENOUSLY INJECTED SUCROSE OF THE KIDNEYS. PAUL KLEMPERER.

In routine examinations of autopsy material of the past two years it was noted that a most striking vacuolation of the cytoplasm of the epithelium of the pri-

mary convoluted tubules of the kidneys was being encountered much more frequently than in preceding years. In an investigation to determine whether any therapeutic procedure might have been responsible for this conspicuous alteration it was found that every one of the patients had received one or more intravenous injections of 50 per cent sucrose solution. These observations are not original, because identical findings in animal experiments have been reported by Lamy, Mayer and Rathery and by Helmholtz, who noted also similar changes in a case in man. The object of the demonstration is to call attention to these striking changes, which have been found frequently and are puzzling.

DISCUSSION

THEODORE BAUER: When I saw the pictures presented by Dr. Klemperer, I remembered the little spots frequently found in all kinds of kidneys; they have been described by Stoerk. They resemble the cortical cells of the adrenal by their big size, their vacuolated protoplasm and their plantlike appearance, so that Stoerk tried to explain, in opposition to Grawitz, the hypernephroma not as a derivative of adrenal rests but, corresponding to these large kidney cells, as nephrogenic tumor. Sometimes one can see only a few cells showing this peculiar degeneration; sometimes, quite a group of tubules with the same changes. The vacuolation may be caused by deposition of lipid or of glycogen. I wish only to draw attention to the point that these peculiar cells, resembling the cells of the cortex of the adrenal gland, caused in the cases just demonstrated by sucrose, follow Stoerk's theory as to the base from which the Grawitz tumor (hypernephroma) cells originate.

IRVING GRAEF: Were these cells observed in normal kidneys?

PAUL KLEMPERER: Years before in routine material I probably observed similar changes, because one sees vacuolation in some severe infections—for instance, in chronic dysentery. Vacuolar degeneration is not too uncommon. It was the frequency with which one encountered this change which was so striking that one had to look for some cause. I think the cause was the sucrose injected, not only because injection of sucrose was found in our cases but also because in experimental investigation an exactly identical change had been found. I want to emphasize the fact that these vacuoles are not lipids nor are they due to deposition of glycogen.

IRVING GRAEF: The significance of my question had to do with the fact that Dr. Klemperer showed pathologic kidneys. Dr. Klemperer may recall a case I have been studying, which was referred to me through Dr. Jacob Werne. It concerned a child who had renal insufficiency for no apparent reason and who at autopsy had markedly swollen kidneys, with hemorrhage into the pelvis and around the pelvis, and, in addition, microscopic thrombosis of the renal vein. The etiologic explanation of this thrombosis was never established. In addition to these abnormalities, the tubules of the kidney everywhere exhibited the same change which Dr. Klemperer showed; the nuclei were normal. I thought there might have been some disturbance in the metabolism or in the excretion of urine which promoted storage of a substance that would ordinarily diffuse out, and I too found that there was no lipid in the epithelium. The point which perplexed me was: Could there be storage of a polysaccharide like sucrose in a normal epithelial cell to the extent which was demonstrated? Must renal insufficiency be present to promote such storage, or some local change in function which would promote such storage? I have had the opportunity of examining the kidneys from animals which had received large amounts of inulin, which ordinarily diffuses through the glomerular membranes, and in these animals I have never seen anything to compare with this change. I wonder whether Dr. Klemperer feels that a local disturbance is necessary to promote such a tendency toward storage of sucrose.

AMOUR F. LIBER: Dr. Klemperer's paper will undoubtedly be extremely enlightening. Just a few days ago I examined slides of a kidney from a child

who had died after prolonged diarrhea, and I recall now that the kidney, which presented no other gross or microscopic lesion, showed a diffuse vacuolar condition of the epithelium of the convoluted tubules, which, it seems to me, must have corresponded to the picture which Dr. Klemperer showed. The child had not received sucrose, which has not been used at the Lincoln Hospital currently, but had received large amounts of dextrose intravenously and perhaps saline solution, but certainly dextrose. I wonder whether this might not be a phenomenon of water accumulation.

PAUL KLEMPERER: In regard to the question whether the vacuoles contain sucrose, I cannot say that they do not, but what is known of the excretion of sucrose speaks against it. Sucrose is excreted by the normal kidney within seventy-two hours quantitatively. Dextrose in very high, 50 per cent, concentrations, also produces vacuolation, though not as striking vacuolation as in these cases. Less concentrated solutions apparently do not produce change.

In regard to Dr. Graef's question: Some of the patients were perfectly normal as far as renal function was concerned. The same feature was shown in a case of subacute bacterial endocarditis with cerebral embolism in which sucrose had been injected. There was also a case of heart failure due to myocardial fibrosis in which there was no renal insufficiency, and the same picture was found. Moreover, the experiments made by Helmholtz showed an identical picture in normal rabbits. I cannot recall exactly the case to which Dr. Graef called my attention, but I may mention 2 cases of renal insufficiency in which there was vacuolation of the severest type with no suspicion of an injection of sucrose, because sucrose therapy was not known at that time. The histologic picture was different. The tubular cells were destroyed to a much greater extent than in the instance reported here, in which the epithelial cell seems intact and shows only striking vacuolation.

PATHOLOGIC CHANGES FOLLOWING THERAPEUTIC HYPERTHERMIA. LOUIS LICHTENSTEIN (by invitation).

A description is given of changes observed at necropsy in a case of uncontrollable hyperpyrexia (109 F.) ensuing on hyperthermic treatment for arthritis of the finger joints. The hyperpyrexia (which developed in the course of the third of a series of treatments) was associated with coma, hemiplegia and respiratory failure, and the subject died about thirty-five hours after the fever was initiated. In this case the pathologic changes were as follows: (1) multiple punctate hemorrhages and necrobiosis in the gray matter of the cerebral cortex; (2) hemorrhage in the left internal capsule; (3) thrombosis of venules and capillaries in the cerebral cortex and internal capsule; (4) cerebral congestion and edema; (5) infarction of kidneys and spleen; (6) marked hepatic degeneration and edema; (7) pulmonary congestion, hemorrhage and edema.

The observations in this case are correlated with, and discussed in relation to, the observations at autopsy in 9 other instances of fatal fever therapy reported within the past few years. Certain of the features presented—notably the vascular lesions—have not hitherto been described in connection with fatalities following fever therapy. Specifically, there seems to be no previous description of the thrombosis of venules and capillaries in the hemorrhagic portions of the brain or of the infarcts in the kidneys and spleen, which apparently were the result of focal necroses of small arterial branches in these organs.

The principal complications and sequelae of hyperthermia, and especially its effects on the brain, blood vessels and liver, are indicated.

DISCUSSION

MAURICE N. RICHTER: I should like to ask whether the vacuoles in the liver contained eosinophilic bodies. From the photograph they resembled the

structures described years ago by Mallory and more recently by Pappenheimer and Hawthorne (*Am. J. Path.*, 12:625, 1936), and they can be found in a rather high percentage of livers in routine examination.

LOUIS LICHTENSTEIN: No eosinophilic bodies were noted microscopically.

ACUTE POSTOPERATIVE ENTEROCOLITIS: A STUDY ON THE PATHOLOGIC NATURE OF SHOCK. ABRAHAM PENNER (by invitation) and ALICE BERNHEIM (by invitation).

This paper will be published in full in a later issue of the ARCHIVES.

CHICAGO PATHOLOGICAL SOCIETY

KATHARINE M. HOWELL, *President*

Regular Monthly Meeting, March 13, 1939

EDWIN F. HIRSCH, *Secretary*

FIBROSARCOMA OF SOFT TISSUE WITH REGIONAL CONCENTRIC ABSORPTION OF BONE NOT DUE TO THE TUMOR. ORMAND C. JULIAN.

The clinical and pathologic details of a slowly growing fibrosarcoma of the soft parts of the forearm are reviewed. The history of the disease from the appearance of the tumor until the amputation of the arm extended sixteen years. The patient had no metastases nine months after the amputation. An unusual feature was the concentric absorption of bone in the radius and ulna which resulted in pathologic fractures. These changes were present at least four years before gross or microscopic evidence of extension of the tumor into the bone was found. The histologic appearance of the tumor tissues was not characteristic of sarcoma. However, regional metastases to the skin of the forearm indicated malignancy.

DISCUSSION

D. H. PHEMISTER: The behavior of the soft tissues was peculiar, and the diagnosis of sarcoma was not simple, because of the long duration of the disease. The disturbance in the bone was an external concentric absorption of the radius and then the ulna. When the radius was first exposed, there was no evidence of tumor in the periosteum. Later the tumor tissues were in the bone. It seems to be a tumorous disease of long duration in the soft parts with spread into the bone.

O. SAPHIR: I have seen several tumors like this, arising in or near the periosteum. Is there evidence that this is a slowly growing neurofibroma? The neurofibroma is radioresistant. Later the growth became sarcoma with invasion of the bone.

O. C. JULIAN: No special stains for the tissues of neurofibroma were made, but palisades of cells were not a special feature. There were no metastases.

KODACHROME FILMS FOR TEACHING PATHOLOGY. S. A. LEVINSON, W. O. BROWN and J. R. THOMPSON.

The advantages of Kodachrome films of fixed and unfixed pathologic tissues for teaching purposes were illustrated and discussed.

HISTOLOGY OF THE PITUITARY OF THE WHITE RAT AFTER INJECTION OF AN ESTROGEN. ARTHUR WEIL and BERNHARD ZONDEK.

Injections of dimenformon (estradiol benzoate) into white rats twice each week over long periods produced the following changes in the pituitary: With

120,000 mouse units, administered during nine to twelve weeks, the anterior lobe was enlarged to about one and a half times its normal size. There was mild swelling of the cells of the three different types. The proportion of eosinophilic cells was 30 to 35 per cent as compared with 38 to 32 per cent in controls given injections of olive oil. With 600,000 mouse units, injected during seventeen to thirty weeks, the anterior lobe was enlarged to about twice its normal size. There was a mild increase in its vascularity. In all of the three types of cells, which were moderately swollen, the Golgi apparatus was markedly enlarged and contained a coarse granular debris. The proportion of eosinophilic cells was diminished to 21 to 28 per cent. With 780,000 mouse units, injected during thirty-two to sixty-three weeks, the anterior lobe was three to four times its normal size and attained a maximal weight of 100 mg. The interlobular cleft was markedly widened. There was a marked increase in vascularity, which in some rats led to intralobular hemorrhages and death. The cells of all the three types were swollen, the Golgi apparatus was maximally dilated, and most of the chromophils were without granules. The pars intermedia and the pars nervosa were not directly affected by the injected estrogenic drug. In the last two groups they were compressed by the enlarged anterior lobe. In the cases in which the changes were advanced the pars nervosa had a loss of cells and an increase in glia fibers. Compression of the hypothalamus led to atrophy of the median eminence and of the supraoptic nuclei. Compression of the anterior cerebral arteries was followed by thrombus formation and softening in the frontal lobes.

PRIMARY FIBROMYXOMA OF THE HEART. HOWARD G. BENJAMIN.

All tumors of the heart are rare, but primary tumors are much less frequent than secondary tumors, the ratio being about 1:16. Secondary tumors, observed in 0.5 per cent of 40,000 necropsies, affect the right side of the heart more frequently than the left. Primary tumors, observed in 0.03 per cent of 40,000 necropsies are more commonly benign than malignant, the ratio being about 3:1. They involve the left side of the heart more often than the right. The most common primary tumor is the myxoma (including fibromyxoma, elastomyxoma and other types), which comprises about 45 per cent of all primary cardiac tumors. It occurs at any age and about equally with respect to the sexes. The left auricle, especially the interauricular septum in the region of the fossa ovalis, is the usual site. The tumor frequently forms a pedunculated intra-auricular mass, which may produce a ball valve obstruction of the mitral orifice. The symptoms and signs of any cardiac tumor, primary or secondary, depend on the size and the location of the growth with special reference to the conduction mechanism of the myocardium and to the orifices of the valves and of the great vessels entering the heart.

A white youth aged 17 complained of increasingly severe dyspnea and of generalized weakness. Hemoptysis, fainting spells and edema of the ankles occurred. His illness followed an infection of the upper respiratory tract and lasted three weeks. He had dyspnea, cyanosis, an apical presystolic thrill and murmur, an apical systolic murmur transmitted to the axilla, an accentuated pulmonic second sound, a pulse rate of 132 with a deficit of 12, and signs of heart failure. Death occurred suddenly eleven hours after he had entered the hospital. Autopsy demonstrated a pedunculated cellular fibromyxoma of the left auricle, producing ball valve obstruction of the mitral orifice and the changes of cardiac decompensation, such as hydrothorax, hydropericardium, pulmonary edema and passive hyperemia of the lungs, liver and spleen. Microscopic examination revealed no evidence of rheumatic or other disease of the heart.

DISCUSSION

H. G. BENJAMIN: Metastatic growths of the heart are usually in the right ventricular tissues, probably owing to the vascular distribution.

Book Reviews

Manual of Veterinary Bacteriology. Raymond A. Kelser, D.V.M., A.M., Ph.D.
Third edition, thoroughly revised. Cloth. Pp. 640, with 93 illustrations.
Price \$6. Baltimore: Williams & Wilkins Company, 1938.

That Kelser's Manual has proved a useful addition to the limited list of satisfactory veterinary textbooks is attested by the fact that the present volume is the third edition in ten years.

The contents are presented in well ordered sequence. After a concise introductory chapter on the history of bacteriology, the author takes up the morphology, physiology and classification of bacteria. The classification is that proposed by the Society of American Bacteriologists, as incorporated in the 1934 revision of Bergey's Manual.

Chapters 3 to 7, inclusive, deal with the microscope, sterilization, preparation of culture mediums, methods of artificial culture and the staining and microscopic study of bacteria. Next, the basic principles of immunization and hypersensitivity are described. The tuberculin reaction, however, is barely mentioned. The importance of the tuberculin test in veterinary medicine leads one to expect at least a brief discussion of the theories concerning this phenomenon. Nor are references to literature in which the student might find this information given.

The chapter on bacterial variation is competently written; the impressive list of references indicates that the recent literature has been surveyed. The dissociation of *Mycobacterium tuberculosis* should have been included in the consideration.

Twenty chapters are then devoted to the pathogenic bacteria of etiologic significance in diseases of the lower animals. The pathogens responsible for important infectious diseases of human beings are not described, since the author considered such bacteria outside the scope of the text. Generally speaking, the respective species of bacteria are adequately considered for classroom purposes, but there are some shortcomings. The most important of these is the failure to bring portions of the text into conformity with the more recent literature. For instance, exceptions might be taken to the statement that a number of strains of *Erysipelothrix rhusiopathiae* "will produce small amounts of acid from glucose and lactose" when the data available indicate that all strains of this organism produce acids from these carbohydrates. Although the salient facts concerning *Brucella abortus* are adequately considered, the pathogenicity of *Brucella suis* (except for guinea pigs) should have been stressed more. The fact that this organism occasionally produces lesions in swine and the possibility that employees of slaughterhouses may contract brucellosis from infected carcasses of swine are of importance to the public health and should have been discussed at least briefly. In the consideration of *Actinomyces necrophorus*, exception should be taken to the statement that this organism is the cause of so-called lip and leg ulceration of sheep. Observations by competent investigators (1934 and before) indicate that the lesions of the mouth, at least, are due to a filtrable virus, with *A. necrophorus* playing the role of a secondary invader. In fact, the evidence available raises doubts whether or not *A. necrophorus* is ever the primary factor in many of the lesions in which it is found.

In the section on Myco. tuberculosis it is stated that tuberculosis of dogs and cats is usually due to the human type of organism; as a matter of fact, dogs are probably equally susceptible to the bovine organism, and the type of infection acquired depends on the circumstances of their exposure. Whether cats are susceptible to the human type of infection is problematic. In the cases of tuberculosis of the cat reported, the disease has been due without exception to the bovine organism.

Among the methods suggested for the isolation of *Myco. tuberculosis* the newer procedures are not mentioned, and in considering the cultural requirements of the bovine form of the organism it is inferred that a glycerinated medium is satisfactory. Experience in isolating this organism from the tissues of naturally infected cattle indicates definitely that most bovine strains of *Myco. tuberculosis* are nonglycerophilic. The statement that in tuberculous chickens pulmonary involvement is quite infrequent is likewise at variance with many observations. In view of the marked reduction of tuberculosis in cattle during the past decade, one might well disagree with the author in his opinion that the bovine type of *Myco. tuberculosis* is the common cause of tuberculosis in swine. The reports of several investigators indicate that the avian type of the organism is responsible for a large proportion of the tuberculous disease in swine. A discussion of heterologous sensitization to tuberculin would also have been of value.

Four chapters are devoted to the pathogenic fungi, and there are 53 well written pages on the protozoa, including the technical methods best suited to the study of these organisms. It is regrettable, however, that *Trichomonas foetus* was not included.

A chapter deals with the various filtrable viruses. This chapter contains a large amount of information on an increasingly important group of animal diseases. The subject is ably presented, and the conclusions are conservative.

The last 4 chapters deal with practical serologic tests and other methods for clinical examination of the blood. A consideration of the blood of the domestic chicken is not included. There are also descriptions of methods for the preparation of biologic products used in veterinary medicine and of the standard methods for bacteriologic examination of milk and water.

The index is adequate. A check of 30 bibliographic references taken at random revealed a few errors of minor importance. The size of the edition has been increased by 88 pages.

While this book has certain shortcomings, the desirable qualities outweigh any deficiencies or errors. The new edition merits a warm reception by teachers and students of veterinary medicine.

Les meningo-neurobrucelloses. Henri Roger and Yres Poursines. Paper. Pp. 248. Price 65 francs. Paris: Masson & Cie, 1938.

The senior author of the book is an outstanding authority on brucellosis as it affects the nervous system. Since 1923 he and his collaborators have published twenty-seven papers on this subject. The increasing number of reports dealing with effects of brucellosis on the nervous system published in recent years indicates that such forms of the disease either passed unobserved or were given little consideration in the past.

The first chapter of the book is devoted to the general conception of the problem of brucellosis in animals and in man. One will not find here a very complete and up-to-date review of this phase of the subject. However, the discussion pertaining to the clinical manifestations of the disease in man is worthy of study. In the succeeding ten chapters the authors have brought together the published observations scattered through the literature and combined them with their own extensive observations, with the result that there is here afforded a comprehensive analysis of the effects of *Brucella* infection on the central and peripheral nervous system. The effects of the disease on each of the principal anatomic parts of the nervous system are discussed in a separate chapter. Due consideration is given to the clinical diagnosis, the prognosis and the therapeutics of nervous forms of the disease. The authors note that the neurologic manifestations seldom if ever appear early in the disease; that the clinical symptoms may present a changing pattern. A large number of the severe forms terminate fatally; others may be observed as mental disturbances of indefinite duration.

The information contained in the book should be a valuable guide to those interested in the neurologic aspects of the disease. It is obvious that one should

not neglect to consider brucellosis when the causes of a disease of the nervous system are sought for in many patients. There is appended a bibliography containing 238 references, most of which pertain to published observations on neuro-brucellosis.

Pathologische Histologie: Ein Unterrichtskurs für Studierende und Ärzte.

Dr. Max Borst, Professor der allgemeinen Pathologie und der pathologischen Anatomie an der Universität München. Third edition. Paper. Pp. 522 with 361 illustrations. Price 75 marks. Berlin: Julius Springer, 1938.

The first edition of this book was published in 1922; the second, in 1926. It grew from 371 pages to the present size. The number of illustrations rose from 240 in the first edition to 275 in the second and to 374 in the present. On the title page mention is made of 361 illustrations but in the text this number of illustrations is increased by 13 insertions. All but 19 illustrations are colored. The introduction is a profound, fascinating philosophic analysis of histopathology and of the part played by the histologist in the practice of medicine. The subject is treated according to anatomic principles, in 10 chapters but for the neoplasms, which are presented collectively in the final (eleventh) chapter, containing 137 pages. The first 2 chapters deal with the organs of circulation and with the blood and blood-forming organs; then follow chapters on the organs of respiration and digestion, on the urinary, genital and nervous system, on organs of locomotion, on the skin and on the glands of internal secretion. Each chapter opens with a concise but clear presentation of the normal histology. While histopathology is stressed, the general pathology and pathogenesis of each subject are considered as exhaustively as the size and purpose of the volume permit. The microscopic changes are correlated with the gross pathologic observations and frequently also with clinical manifestations. The pictures were drawn by W. Freytag from original preparations; they are well chosen and excellently executed. A valuable feature is the thorough textual analysis of minute details of the pictures. Recent developments are discussed. There are no references. The paper and print are good. A well organized index fills 15 pages. The book can be recommended warmly to all students of histopathology.

The Chemistry of Antigens and Antibodies. By J. R. Marrack, D.S.O., M.C., M.D. Paper. Pp. 194, with illustrations. Price 3 shillings. Medical Research Council, Special Report Series, no. 230. London: His Majesty's Stationery Office, 1938.

This monograph is a new edition of special report 194, revised and enlarged. The aim in this, as in the first edition, is to apply physicochemical principles and methods to the study of the nature of immune reactions. The general plan of the book remains the same. It contains, as before, five chapters, each of which has been extensively revised. In the first chapter, on physicochemical considerations, the main change is in the discussion of recent theories of protein structure. Chapters 2 and 3, on the nature of antibodies and the specificity of antigens, respectively, have been considerably enlarged and brought up to date. The most extensive additions have been made to chapters 4 and 5, on the nature of antigen-antibody reactions. This volume, like its predecessor, represents the best attempt this reviewer has seen to apply chemical and physicochemical principles to the solution of immunologic problems. It should prove of great value in stimulating students of immunology to apply physicochemical methods to the study of the mechanism involved in antigen-antibody reactions. There are extensive references to the original literature for those interested in following the advances being made in this fascinating field.

Books Received

CLINICAL BIOCHEMISTRY. Abraham Cantarow, M.D., Associate Professor of Medicine, Jefferson Medical College; Biochemist, Jefferson Hospital. Max Trumper, Ph.D., Clinical Chemist and Toxicologist; formerly in charge of the Laboratories of Biochemistry of the Jefferson Medical College and Hospital. With a foreword by Hobart A. Reimann, M.D., Professor of Medicine, Jefferson Medical College. Second edition, revised. Cloth. Pp. 666. Price \$6. Philadelphia: W. B. Saunders Company, 1939.

THE GENERAL TISSUE AND HUMORAL RESPONSE TO AN AVIRULENT TUBERCLE BACILLUS INCLUDING GROWTH CHARACTERISTICS OF THE ORGANISM. Sol Roy Rosenthal, M.D., Ph.D., Associate in Bacteriology and Public Health. Joint Contribution from the Tice Laboratories of the City of Chicago Municipal Tuberculosis Sanitarium and the College of Medicine of the University of Illinois. Illinois Medical and Dental Monographs, vol. 2, no. 2. Paper. Pp. 184, with 80 illustrations. Price \$2.50. Urbana: University of Illinois Press, 1938.

CRYSTALLINE ENZYMES. THE CHEMISTRY OF PEPSIN, TRYPSIN, and BACTERIOPHAGE. John H. Northrop, Member of the Rockefeller Institute for Medical Research. Cloth. Pp. 176, with 48 illustrations. Price \$3. New York: Columbia University Press, 1939.

ANGINA PECTORIS. NERVE PATHWAYS, PHYSIOLOGY, SYMPTOMATOLOGY, AND TREATMENT. H. R. Miller, M.D., Attending Physician, Sydenham Hospital; Associate Attending Physician, Montefiore Hospital, New York City. Cloth. Pp. 275, with 39 illustrations. Price \$3.25. Baltimore: William Wood & Company, 1939.

FAILURE OF THE CIRCULATION. Tinsley Randolph Harrison, M.D., Associate Professor of Medicine, Vanderbilt University, School of Medicine, Nashville, Tenn. Second edition, revised. Cloth. Pp. 495, with 60 illustrations. Price \$4.50. Baltimore: Williams & Wilkins Company, 1939.

BY-EFFECTS IN SALVARSAN THERAPY AND THEIR PREVENTION WITH SPECIAL REFERENCE TO LIVER FUNCTION. V. Genner. Paper. Pp. 360. Copenhagen: Levin & Munksgaard, 1936.

TWENTY-NINTH ANNUAL REPORT OF THE CHARLES V. CHAPIN HOSPITAL, PROVIDENCE, R. I., FOR THE YEAR ENDING SEPTEMBER 30, 1938. Paper. Pp. 82. Providence: The Oxford Press, 1938.

ACCESSORY ADRENAL CORTICAL TISSUE

ARTHUR A. NELSON, M.D., PH.D.

WASHINGTON, D. C.

An accumulation of accessory adrenal cortical tissues from 19 persons, together with the unorganized state of the literature on this subject, has prompted the writing of this paper. Ten of these 19 specimens of accessory cortical tissue were found in the immediate vicinity of testes; 6 of these 10 specimens were obtained from adults, making this the largest group of the kind reported; 1 specimen was of tumor proportions and apparently the largest mass of this type yet observed in this situation.

Accessory adrenal tissue in the near vicinity of the adrenal was first described by Morgagni,¹ in 1740. Accessory adrenal cortical tissue at a distance from the adrenal was first described by Marchand,² in 1883. Over a period of seven years Marchand found yellow nodules, from 1 to 3 mm. in diameter, in the free edge of the broad ligament near the ovary in 5 newborn and older infants, and in a fetus of 5 months he found a similar nodule on each side between the lower pole of the kidney and the broad ligament; microscopically these nodules had the structure of adrenal cortex. Reports by eight other authors of the finding of accessory adrenal tissue in the broad ligament, in adults as well as in newborn infants, published up to 1900, were listed by Aichel,³ who described several such observations of his own in fetuses and newborn infants.

Chiari,⁴ one year after Marchand, was the first to describe accessory cortical adrenal tissue at a distance from the adrenal in adults. In men of 23 and 34 years and in a woman of 30 years he found accessory nodules below the lower pole of the right kidney, in the vicinity of or lying on the spermatic and ovarian veins; in a woman of 48 years there was a nodule on the right side anterior to the ovarian veins at

From the National Institute of Health.

1. Morgagni, G., cited by Wiesel, J.: *Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Cl. (Abt. 3)* **108**:257, 1899.

2. Marchand, F.: *Virchows Arch. f. path. Anat.* **92**:11, 1883.

3. Aichel, O.: *Arch. f. mikr. Anat.* **56**:1, 1900.

4. Chiari, H.: *Ztschr. f. Heilk.* **5**:449, 1884.

the level of the linea terminalis of the pelvis, and on the left side, two nodules in the broad ligament. All of these nodules varied from submaxillary to pea size. In the same year d'Ajutolo⁵ reported a cherry-sized nodule on the spermatic cord of a newborn infant, just above the internal ring.

The first to describe accessory adrenal cortical tissue in the near vicinity of the testis was Dagonet,⁶ in 1885; in a 21 day old infant he found two accessories, each 3 mm. in diameter, one on the internal spermatic plexus (side not stated) and one between the right testis and the epididymis. Other reported cases of accessory adrenal tissue near or in the testis or ovary will be considered later.

The cases of accessory adrenal cortical tissue reported before 1900 were reviewed by Wiesel⁷ and Aichel.³ Weller⁸ reviewed the 13 reported cases (to 1925) of true renal or hepatic adrenal heterotopia (no adrenal except the inclusion present on that side) and added 4 cases of his own.

Jaffe in his review⁹ and Goldzieher¹⁰ in his book on the adrenals mentioned accessory tissue only briefly.

SYSTEMATIC STUDIES OF OCCURRENCE OF ADRENAL ACCESSORIES

No systematic study of human material with respect to the occurrence of accessory adrenal cortical tissue in all locations where it might be reasonably expected to occur has ever been published. There are a few reports of studies of one particular region in more or less large numbers of cases. Schmorl¹¹ reported finding small masses (three in one case) of adrenal cortical tissue just under the capsule of the right lobe of the liver, close to the adrenal, in 4 of 510 persons examined post mortem within a period of seven months; 2 of these were men of 24 and 56 years and 2 were women of 36 and 37 years; most of the masses were of pinhead size, while the largest was lentil size. Schmorl also found a pea-sized nodule just outside the external inguinal ring on the right spermatic cord of a man 30 years of age.

Concerning the occurrence of accessory tissue in the kidney, Lubarsch¹² briefly stated that he had found adrenal cortical tissue in

5. d'Ajutolo, G.: *Arch. per le sc. med.* **8**:283, 1884.

6. Dagonet, J.: *Ztschr. f. Heilk.* **6**:1, 1885.

7. Wiesel, J.: *Sitzungsab. d. k. Akad. d. Wissensch. Math.-naturw. Cl. (Abt. 3)* **108**:257, 1899.

8. Weller, C. V.: *Am. J. M. Sc.* **169**:696, 1925.

9. Jaffe, H. L.: *Arch. Path.* **3**:414, 1927.

10. Goldzieher, M.: *The Adrenals*, New York, The Macmillan Company, 1929.

11. Schmorl, G.: *Beitr. z. path. Anat. u. z. allg. Path.* **9**:523, 1891.

12. Lubarsch, O.: *Virchows Arch. f. path. Anat.* **135**:149, 1894.

the kidney in 8 of 300 bodies and "much more frequently" along the adrenal and internal spermatic veins. Glynn¹³ found no accessory tissue in 1,500 kidneys. Brites¹⁴ examined the kidneys of 376 bodies and found accessory adrenal cortical tissue in 10; later he found none in 1,100 human kidneys, 1,060 sheep kidneys, 34 goat kidneys and 58 beef kidneys. My own 5 examples were found among 630 persons examined post mortem. McLennan,¹⁵ examining the hernial sacs removed at operation from 700 children (660 of whom were boys), found nodules of adrenal cortical tissue embedded in the walls of 6 of the sacs. These nodules were near, but not attached to, the spermatic cord. He had not seen any such nodules in adults.

The only reported systematic study of the testes for the presence of accessory adrenal cortical tissue is that of Wiesel,⁷ who examined 15 pairs of testes and epididymides from newborn infants and an equal number from children over 1 year of age and adults. In 13 of the newborn infants bits of accessory tissue were found on one side (9 on the right, 4 on the left) and in 5 on both sides; thus, in 23 (76.5 per cent) of the 30 gonads altogether. The most common location was in the connective tissue around the tail of the epididymis. No fully developed accessory tissue was found in the testes of the children and adults, although in the younger persons especially, Wiesel stated, he could find cell masses and strands which had somewhat the appearance of adrenal cortex and which could be thought of as being in a stage of regression.

ACCESSORY ADRENAL CORTICAL TISSUE IN ANIMALS

The occurrence of accessory adrenal cortical tissue in animals has been known since at least 1887, when Canalis¹⁶ found among 40 rabbits 2 adrenal accessories of rice grain size, each on the right side, near the mouth of the renal vein; these were composed of cortical tissue only. Wiesel⁷ found adrenal accessories in about 50 per cent of rats, and more in sexually active than in very young or very old rats. Jaffe⁹ found gross accessories in about 8 per cent of normal rats and in from 20 to 25 per cent after double adrenalectomy; these accessories were always situated near the main glands, and Jaffe could find none in the region of the testis, vas and epididymis. Microscopically he could find accessories on serial section of the retroperitoneal tissues in

13. Glynn, E. E.: *Quart. J. Med.* **5**:157, 1912; *J. Obst. & Gynaec. Brit. Emp.* **28**:23, 1921.

14. Brites, G.: *Folia anat. univ. conimb.* **10**:1, 1935.

15. McLennan, A.: *Surg., Gynec. & Obst.* **29**:387, 1919.

16. Canalis, P.: *Internat. Monatschr. f. Anat. u. Physiol.* **4**:312, 1887.

70 per cent of rats before sexual maturity. Marine and Baumann¹⁷ reported gross accessories in 70 per cent of rabbits after adrenalectomy.

The constant occurrence of adrenal cortical tissue within the genital tract of both the male and the female rabbit has been recently established by Lacassagne and Nyka.¹⁸ In every one of 10 adult male rabbits a nodule of adrenal cortical tissue was found, usually in the adipose tissue around the posteroinferior portion of the head of the epididymis at the level of the junction of the rete testis and efferent ducts; it was on the right side in 5, on the left in 4 and bilateral in 1. In 10 female rabbits serial sections of the adnexae showed a similar structure in all, 8 times on the right side, once on the left, and once bilaterally.

FREQUENCY OF OCCURRENCE OF ACCESSORY TISSUE

A statement which has been frequently quoted, often at second hand and often in various erroneous forms, and which deserves some explanation at this time, is to the effect that Schmorl found accessory adrenals in 92 per cent of all bodies. Such a statement without further qualification might well give rise to the idea that these were accessory adrenals at some distance from the main glands and that one or more such bodies could be found in nearly every cadaver. This statement or references to statements based on it may be found in such current sources of information about the adrenals as Goldzieher¹⁹ and Ewing.¹⁹ In these and in various papers on accessory adrenals eventual reference is made to a paper by Schmorl appearing in volume 9 of the *Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie* for the year 1891. The fact is that in this particular paper¹¹ Schmorl made no such statement. However, in the same number of that journal, in a paper concerning tumors thought to arise from adrenal rests, by Beneke,²⁰ the statement is made that *die "Versprengung" von Nebennierenkeimen findet sich, wie ich von Herrn Kollegen Dr. Schmorl auf Grund einer von ihm aufgestellten Statistik erfahre, bei 92% aller Leichen, meist in der nächsten, aber auch in der weiteren Umgebung der Nebennieren. . . .* (The "scattering" of adrenal rests, as I learned from Dr. Schmorl, on the basis of statistics compiled by him, is this—that adrenal rests are found in 92 per cent of all bodies, usually in the immediate vicinity, but also in the more distant vicinity, of the adrenals.) During the next few years this statement

17. Marine, D., and Baumann, E. J.: J. Metab. Research **1**:777, 1922.

18. Lacassagne, A., and Nyka, W.: Compt. rend. Soc. de biol. **118**:1406, 1935; **121**:95, 1936.

19. Ewing, J.: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1928.

20. Beneke, R.: Beitr. z. path. Anat. u. z. allg. Path. **9**:440, 1891.

was misquoted on so many occasions that Rossa²¹ asked Schmorl himself about it and discussed it and the misquotations of the latter's paper. According to Rossa, Schmorl found accessory adrenal tissue in 92 per cent of "a large number" of bodies, nearly all of adults; included were those accessories in the immediate vicinity of the adrenal, in the strands of the solar plexus and along the adrenal and spermatic veins, but not those in the renal cortex or the under surface of the liver; in all doubtful cases microscopic examination was done. The important fact is that no figure for each separate location was given; in all probability, the great majority of the 92 per cent were accounted for by those in the immediate vicinity of the adrenal, and accessory adrenal cortical nodules at any distance from the adrenal will undoubtedly be found to occur in far less than 92 per cent of bodies of all ages.

ACCESSORY TISSUE WITHIN THE TESTIS OR OVARY

In all the reported cases of accessory adrenal cortical tissue in the vicinity of the testis and epididymis, the tissue has been situated in the mediastinum testis, the tunics of the testis, the connective tissue between the testis and the epididymis, near the epididymis or within the epididymis (a few cases). No one has yet reported a case of adrenal tissue situated deep within the testis, and only R. Meyer²² has reported an instance in which the accessory tissue appears to be underneath the tunica albuginea; this was a small nodule in a fetus. Two of Wiesel's²³ accessory nodules in newborn infants were within the epididymis, among the tubules; Kirkbride²⁴ illustrated an accessory nodule among the ducts of the epididymis in a newborn infant; Marsella²⁴ did the same for a man 37 years of age.

In the ovary, adrenal cortical tissue was first reported by Lodi,²⁵ in 1902. He found nodules from pea to hazelnut size in the ovaries of a woman who had been pregnant fourteen times. Some of the nodules had masses of fibrin in their centers, making it probable that these were masses of lutein cells. Marchetti²⁶ found, among 1,200 autopsies, what he thought were 2 small masses of adrenal cortical cells near a cyst in an ovary from an adult woman. Varaldo²⁷ in the same year reported rests, 5 to 7 mm. in diameter, in the ovaries of 3 women from 30 to 41 years of age (1 in each instance). These were

21. Rossa, E.: *Arch. f. Gynäk.* **56**:296, 1898.

22. Meyer, R.: *Ztschr. f. Geburtsh. u. Gynäk.* **71**:221, 1912.

23. Kirkbride, M. B.: *Arch. f. Entwicklgsmechn. d. Organ.* **32**:717, 1911.

24. Marsella, A.: *Arch. ital. di urol.* **11**:281, 1934.

25. Lodi, M.: *Arch. ital. de biol.* **37**:486, 1902.

26. Marchetti, G.: *Virchows Arch. f. path. Anat.* **177**:227, 1904.

27. Varaldo, F.: *Arch. di ostet. e ginec.* **11**:725, 1904.

also near cysts. Jessup²⁸ found a circular group of cells in the cortex of an ovary from a 16 day old girl. The outer cells resembled the zona glomerulosa of the adrenal cortex; the inner, the zona fasciculata. There is no illustration and no mention of a fat stain. Berger,²⁹ in reporting a case of pheochromine tissue in the ovarian hilus of a newborn infant, stated briefly that he had seen adrenal cortical tissue three times in the testis and thirteen times in the ovary; details of the exact locations he did not give.

ADRENAL TISSUE IN HEAD REGION

A unique case is that reported by A. W. Meyer,³⁰ who found an encapsulated nodule, 0.8 by 1.5 cm., attached to the spinal portion of the eleventh cranial nerve. Microscopically it had the appearance of adrenal zona glomerulosa and zona fasciculata with medulla-like cells in the center. The capsule contained masses of chromaffin cells and a bundle of striated muscle.

TUMORS ARISING FROM ACCESSORY ADRENALS

Following the idea of Grawitz³¹ in 1883 that renal hypernephroma arises from accessory adrenal tissue, investigators similarly described tumors in other locations, especially tumor in the ovary. The most thorough articles on this subject were contributed by Glynn.¹³ He accepted only 1 case, that observed by Bovin,³² as an instance of a tumor arising from an adrenal rest. In this case a tumor in the broad ligament of a woman of 28 years caused amenorrhea and the development of a beard. Menstruation returned after removal of tumor; the beard persisted. Kolodny³³ reported the case of a 37 year old woman whose amenorrhea and hirsutism were relieved by removal of a 1,250 Gm. retroperitoneal epigastric tumor, which was invading the stomach; histologically the tumor was composed of wide anastomosing strands of large clear cells containing abundant lipid droplets and resembling the zona fasciculata of the adrenal. However, the patient died seven months after operation, with pulmonary metastases; an autopsy was not mentioned. Saphir and Parker³⁴ reported another case of adrenal virilism in a girl of 15 years; here it was thought that a small nest of cells in the right ovary, resembling adrenal cortex, might have been responsible.

28. Jessup, D. S. D.: *Proc. New York Path. Soc.* **13**:67, 1913.

29. Berger, L.: *Arch. d'anat. micr.* **32**:315, 1936.

30. Meyer, A. W.: *Anat. Rec.* **12**:43, 1917.

31. Grawitz, P.: *Virchows Arch. f. path. Anat.* **93**:39, 1883.

32. Bovin, E.: *Nord. med. Ark. (sect. 1)*, 1908, vol. 41, no. 15.

33. Kolodny, A.: *J. A. M. A.* **102**:925, 1934.

34. Saphir, W., and Parker, M. L.: *J. A. M. A.* **107**:1286, 1936.

PERSONAL OBSERVATIONS

I wish to report 10 examples of accessory adrenal cortical tissue (1 of tumor proportions) in the near vicinity of the testis, 5 of accessory tissue under the capsule of the kidney, 2 of such tissue on the spermatic

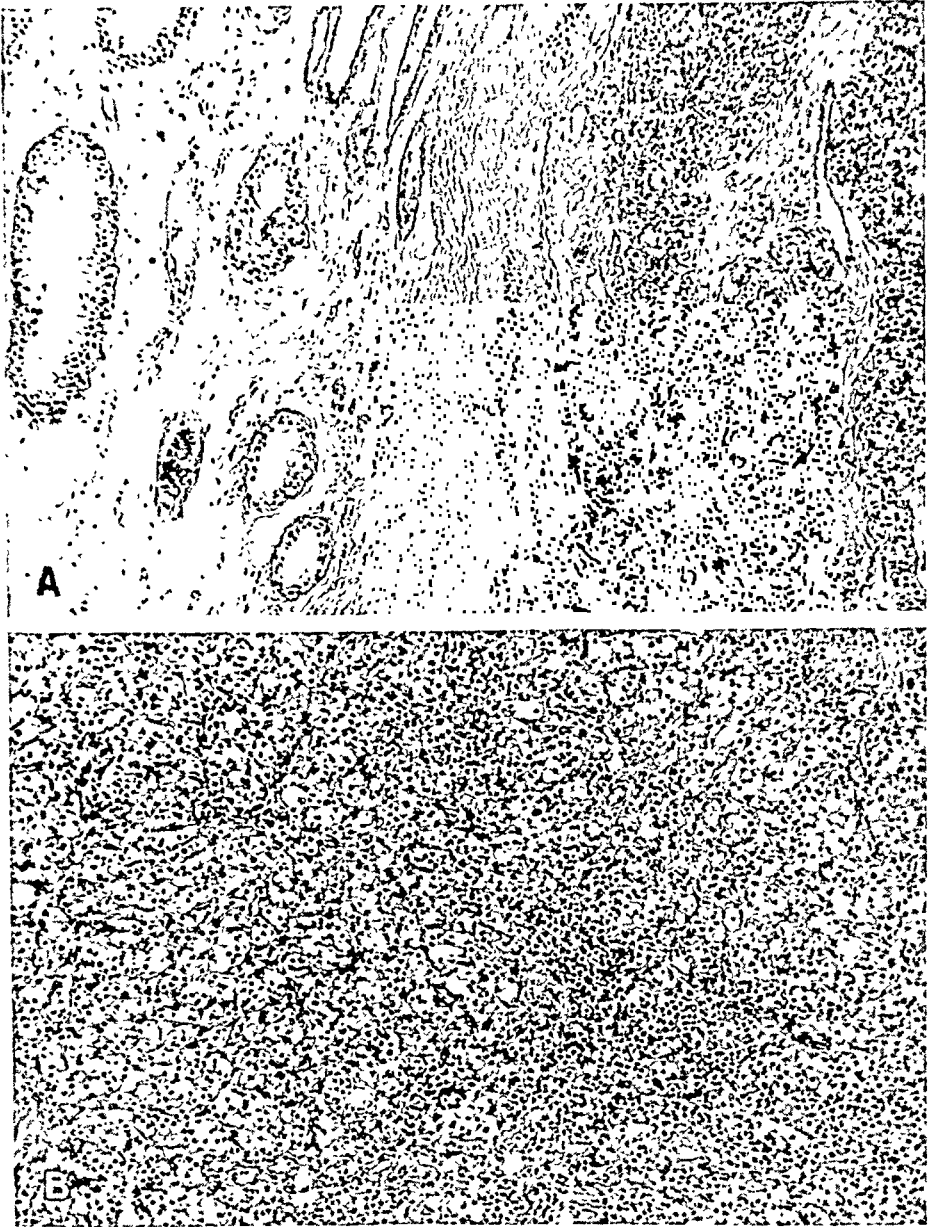


Fig. 1 (case 1).—*A*, section showing the closeness of the adrenal cortical nodule to the testicular tubules; $\times 75$. *B*, interior of tumor; $\times 75$.

cord and 1 of such tissue in the broad ligament. The first, an accessory adrenal cortical nodule, 1.7 cm. in diameter, enclosed in the tunica albuginea of the testis, is presented through the courtesy of Dr. G. L.

Berdez, pathologist of St. Mary's Hospital, Duluth, Minn. This nodule appears to be the largest of its type yet observed in this situation.

CASE 1.—A Jew, a man 35 years of age, noted a small tumor of the right testis three years prior to examination; the nodule did not grow, but he became worried about it; his father had died at the age of 36 from a malignant growth of the testis. Physical examination showed a firm smooth mass, about 1 cm. in diameter, at the lower pole of the right testis; this mass seemed to be a part of the testis, but its outline was easily determined; it was not painful to palpation. At operation it was found to be encapsulated and was easily shelled out. Gross examination showed an almost spherical tumor, 1.7 cm. in diameter, covered by a thin fibrous capsule. On cut section the tumor tissue was of moderately firm consistency and bright yellow. Microscopic examination showed that the tissue was formed by cords of large and medium-sized polyhedral cells with foamy

TABLE 1.—*Accessory Adrenal Cortical Nodules Near Testes*

Case	Age, Yr.	Cause of Death	Side	Size, Mm.	Comment	Atrophy of Testis
2	44	Carcinoma of prostate..	R	3	Moderate
3	42	(Operative specimen)....	R	2	Very marked
4	New-born	(Premature, 1,550 Gm.)..	?	1 each (2)	None
5	New-born	(Stillborn, 3,270 Gm.)....	?	1.5	None
6	63	Carcinoma of bladder...	Bilateral	R—1 × 2 L—1.5 × 3	Moderate regression	Marked
7	5	Lymphatic leukemia.....	?	1	Slight regression	None
8	71	Osteogenic sarcoma.....	L	0.5 × 1	Marked regression	Moderate
9	2½	Hydronephrosis.....	?	0.5	Moderate regression	None
10	61	Pernicious anemia.....	L	1 × 2	Marked regression	Marked
11	47	Carcinoma of mouth....	?	1.5 × 2	Moderate regression	Moderate

reticulated cytoplasm and nuclei and nucleoli of moderate size. There was a very marked resemblance to adrenal cortex. Groups of cords were separated by thin and thick fibrous septums. Only few mitoses were seen. Sections stained with scarlet red showed the cells heavily loaded with fat; the sections grossly were of a deep red color. The pathologic diagnosis was "cortical adrenal adenoma of the testis." Figure 1 *A* shows how closely the tumor tissue approached the testicular tubules, and figure 1 *B* gives a better view of the tumor tissue.

The remaining examples are from autopsy and surgical material received at the National Institute of Health and from material obtained at autopsies made by myself at the University of Minnesota. Sections of the testes and epididymides were made without regard to the possible presence of adrenal rests, and for this reason it is difficult to give the exact frequency of these bits of accessory tissue; this would require serial sectioning. None of these nodules were noted grossly. The 5

cases of accessory tissue under the capsule of the kidney and that of a nodule in the broad ligament were encountered among 630 autopsies made by me; here, of course, the surface of each kidney was inspected according to routine and a definite figure for the incidence of accessory

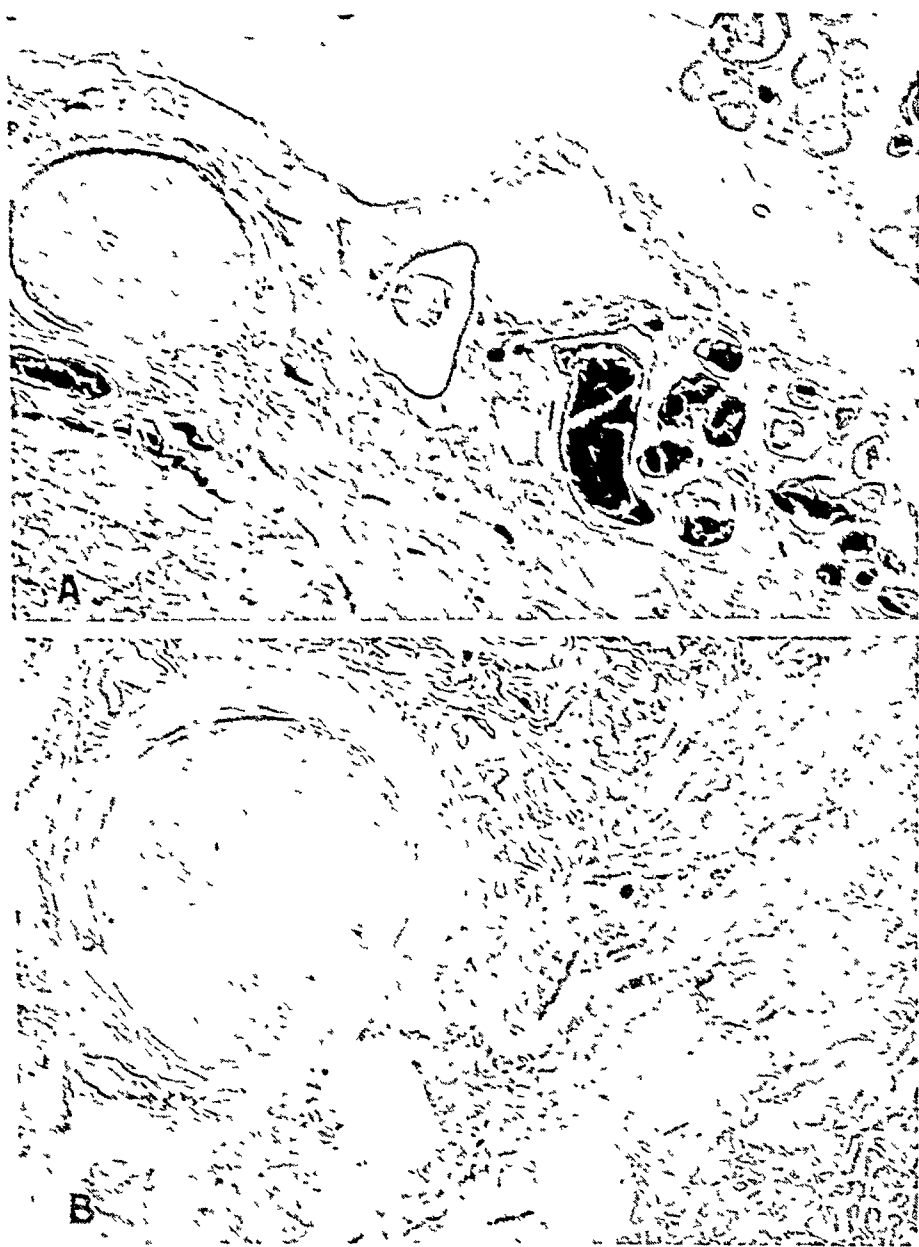


Fig. 2.—*A*, adrenal cortical nodule adjacent to testis (case 3); $\times 9$. *B*, adrenal cortical nodule adjacent to testis (case 2); $\times 8$.

nodules can be given; as previously mentioned, it agrees fairly well with the figures reported in the literature.

In all 10 cases listed in table 1 (case 1 is not included) the accessory adrenal cortical nodule was found in one general location, namely,

either within or just outside the tunica albuginea and either behind or just above or below the rete testis (fig. 2*A* and *B*). In 4 of the 10 cases the nodule showed microscopically the typical structure of

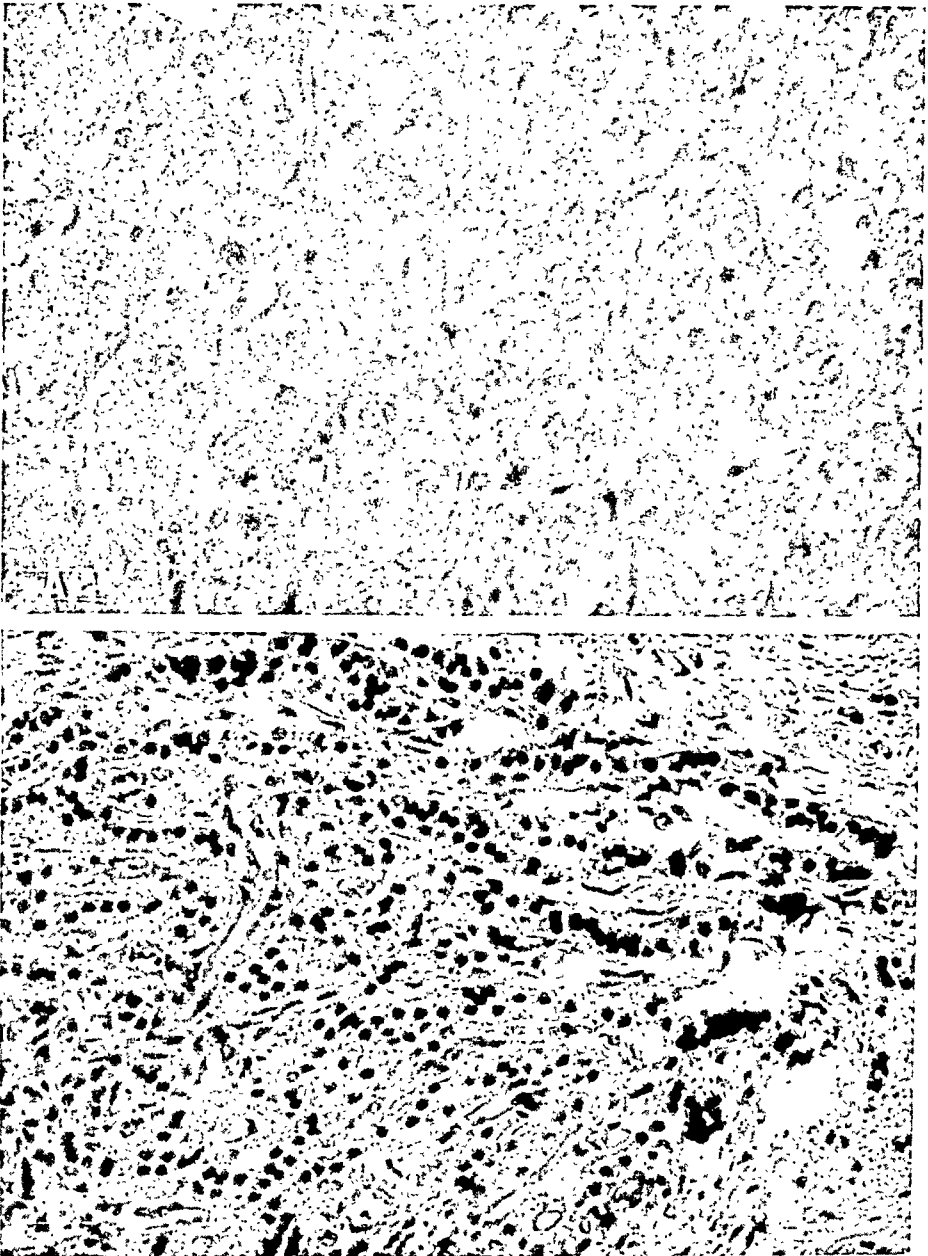


Fig. 3.—*A*, high power view of accessory nodule, showing appearance identical with that of zona fasciculata of adrenal cortex (case 2); $\times 220$. *B*, adrenal cortical nodule undergoing regression (case 9); $\times 180$.

adrenal cortex (fig. 3*A*); in the other 6 instances there were fibrotic and atrophic changes varying from slight to marked in degree; such changes are in all probability retrogressive, and an example of the

moderate type is shown in figure 3B. (This was the smallest accessory nodule observed; more marked retrogressive changes were seen in larger nodules.) The cells lose more or less of their foamy appearance and of the reticulation of their cytoplasm, are smaller and appear much like the cells usually seen immediately under the capsule of the normal adrenal. They still maintain their arrangement in cords, which serves to distinguish them from the masses of interstitial cells often found in the tunica albuginea³⁵; in the latter the cells are much less regular in shape and arrangement, and the masses are usually closely associated with nerve fiber bundles and are seldom as large as the masses of adrenal cortical cells.

TABLE 2.—*Accessory Adrenal Cortical Nodules Under Renal Capsules*

Case	Age, Yr.	Sex	Cause of Death	Comment
12	54	F	Hypertension	Each of these nodules was immediately under the capsule, on the superior pole of the kidney; they were from 1 by 2 to 1.5 by 3 mm. in size. In case 15 two nodules were present in one kidney
13	28	M	Medulloblastoma	
14	83	F	Carcinoma of breast	
15	20	M	Cirrhosis of liver	
16	57	M	Cerebral trauma	

TABLE 3.—*Accessory Adrenal Cortical Nodules on Spermatic Cords*

Case	Age, Yr.	Side	Size, Mm.	Comment	Atrophy of Testis
17	50	R	2	Noted on cord during herniotomy; verified microscopically	?
18	22	L	1 × 1.5	Noted on microscopic examination of tissues from herniotomy	Marked

In addition to the examples listed in the tables I have found at autopsy a 3 mm. yellow nodule of adrenal cortical tissue near the free edge of the left broad ligament in a 62 year old woman who died of bronchial asthma; the nature of the tissue was verified microscopically, as it was in the other 18 tabulated cases.

SUMMARY

There are here reported 19 cases of accessory adrenal cortical tissue; in 10 of these the accessory tissue was found between the testis and the epididymis; in 1 it was at the lower pole of the testis and was of tumor proportions; in 5 it was under the renal capsule; in 2 it was on the spermatic cord, and in 1, in the broad ligament. Fifteen of these 19 persons were adults. A review of the literature is also given.

35. Nelson, A. A.: *Am. J. Path.* **14**:831, 1938.

ACUTE POSTOPERATIVE ENTEROCOLITIS

A STUDY ON THE PATHOLOGIC NATURE OF SHOCK

ABRAHAM PENNER, M.D.

AND

ALICE IDA BERNHEIM, M.D.

NEW YORK

The chance observation of the sloughing of intestinal mucous membrane following radical mastectomy in a patient with severe diabetes led us to inquire into the factors and mechanisms responsible for this phenomenon. With this in view we reviewed the postmortem records of the Mount Sinai Hospital for similar cases of acute diphtheritic enteritis and, in addition, searched the literature for similar observations. The serious nature of this complication, as well as the importance of the mechanism involved in its production, leads us to report our findings.

HISTORICAL REVIEW

The search of the literature revealed but few reports made in sufficient detail to identify clearly the nature of the process dealt with. The earliest report of a case which we were able to find is that of Finney.¹ This surgeon reported a case of benign pyloric obstruction for which he performed pylorotomy and gastroenterostomy. The patient apparently was well for the first ten postoperative days. Then bloody diarrhea developed, which led to a fatal outcome after five days. The postmortem examination, made by Dr. Simon Flexner, showed that there was no evidence of peritonitis but that the bloody diarrhea was due to diphtheritic enteritis involving the terminal ileum and colon down to and including the rectum, but with diminishing intensity distally. The report contains no microscopic description of the lesion.

Terrier and Hartman,² in their monograph on gastric surgery, published in 1899, reported the occurrence of diarrhea in 7 of the 35 cases reported in detail in their text. This diarrhea occurred after gastroenterostomy, pylorotomy or gastrectomy. However, the clinical and pathologic data are so scant that it is impossible to evaluate the cases

From the Laboratories of the Mount Sinai Hospital.

1. Finney, J. M. T.: *Bull. Johns Hopkins Hosp.* 4:53, 1893.

2. Terrier, F., and Hartman, H.: *Chirurgie de l'estomac*, Paris, G. Steinheil, 1899.

properly. Three years later Riedel³ published a report entitled "Ueber Darmdiphtherie nach schweren Operationem bei sehr geschwächten Kranken." He described 5 cases in which an anatomic diphtheritic lesion of the intestine occurred postoperatively. He recognized the anatomic resemblance of the intestinal lesions to those produced by mercury poisoning and was particularly interested in pointing out that the suture material used in the operations had been thoroughly rinsed free from any significant amount of this substance. His first case occurred in 1899. The lesion involved the colon, apparently mainly in the region of the cecum. The latter was dark red and edematous and showed focal areas covered with a gray to grayish green membrane. These changes occurred after hysterectomy for fibroids in a very anemic, cachectic woman of 47 years, who "went into collapse" twenty-four hours after the operation and died four days later. His second case was that of a 52 year old woman on whom he had performed almost total gastrectomy for an enormous growth extending almost to the cardia. Diarrhea developed three days after the operation, and death occurred twenty-four hours later. Autopsy showed the typical lesion involving the lower part of the jejunum, the cecum and the transverse colon, with apparently normal intestine intervening. There was no peritonitis in either of these cases, and Riedel felt that they were not cases of infectious dysentery. His third case was one in which the diphtheritic lesion occurred after a Billroth 1 operation for pyloric stenosis resulting from drinking sulfuric acid with suicidal intent. This patient did not have postoperative diarrhea but died in "collapse" three days after the operation. Autopsy showed a diphtheritic lesion restricted to the ileum. There was no evidence of peritonitis. In his last 2 cases, although the changes presented post mortem were typical, the findings were complicated by the fact that the affected loops of intestine had been manipulated extensively in the operative procedures. Riedel is particularly emphatic in his assertion that preoperative catharsis was not involved in the pathogenesis of the lesion. He felt that the protracted operation, loss of blood and anesthesia, plus the preoperative "weakness" and anemia, united to make the intestine unable to resist its own contents. "This diphtheria of the intestine is nothing more than a superficial necrosis of a poorly nourished mucosa." This analysis represents the first attempt to correlate the preoperative condition and the operative trauma with the occurrence of the diphtheritic intestinal lesion. Four years later Anschütz,⁴ in discussing intestinal disturbances following gastric operations, presented 4 cases of the same type. In these

3. Riedel: *Deutsche Ztschr. f. Chir.* **67**:402, 1902.

4. Anschütz, W.: *Mitt. a. d. Grenzgeb. d. Med. u. Chir.* **15**:305, 1905.

instances the lesion occurred following gastroenterostomy for a benign lesion or after multiple operations. In 2 of the 4 cases postoperative diarrhea never developed. In addition, Anschütz noted 3 cases in which the typical postoperative diarrhea was present but in which the patient survived, so that anatomic confirmation of the lesion was not obtained. In still another case the diarrhea terminated fatally but autopsy was not done. Anschütz has described the lesion as necrotizing, ulcerative ileitis, colitis and proctitis which diminished in intensity distally. He attributed it to the occurrence of putrefaction in the stomach and intestines of a "cachectic" patient.

The occurrence of diphtheritic enteritis was briefly mentioned by Wertheim,⁵ who observed it three times in a series of 500 consecutive cases of radical exenteration of the pelvis for carcinoma of the cervix. The lesion involved the rectum and colon and was invariably associated with a fatal outcome. Rössle⁶ also noted 2 instances in which necrotizing diphtheroid colitis followed a pelvic operation. In one the radical Wertheim operation was performed; in the other, lysis of old pelvic adhesions led to purulent peritonitis, which terminated fatally. He emphasized the role played by medicated enemas in causing local trauma. A similar lesion involving the small intestine and cecum was reported by de Rouville and Roger⁷ in a patient subjected to hysterectomy. In this instance diarrhea appeared on the eighteenth postoperative day and led to a fatal issue four days later. In this case several of the ulcerations extended deeply enough to perforate.

Finsterer⁸ observed acute catarrhal enteritis and colitis following gastric resection in which the entire lesser curvature was removed and only a small portion of the greater curvature remained. Diarrhea developed on the sixth day, and the patient succumbed on the tenth postoperative day.

The first really intensive study of this postoperative complication was made by Bierende.⁹ He made a careful investigation of 7 cases. In 5 of these acute peritonitis was present at the time of operation or supervened shortly thereafter. His patients were aged from 50 to 63 years. The operative procedures included: (1) appendectomy for acute appendicitis complicated by purulent paranephritis with perforation into the pleura and empyema; (2) suturing of a perforated duodenal ulcer, which was then side-tracked by gastroenterostomy; (3) Wertheim's procedure for carcinoma of the cervix; (4) gastric

5. Wertheim, E.: *Die erweiterte abdominale Operation bei Carcinom colli uteri (auf Grund von 500 Fällen)* Berlin, Urban & Schwarzenberg, 1911.

6. Rössle, R.: *Monatschr. f. Geburtsh. u. Gynäk.* **35**:243, 1912.

7. de Rouville, G., and Roger, H.: *Arch. d. mal. de l'app. digestif* **7**:24, 1913.

8. Finsterer, H.: *Deutsche Ztschr. f. Chir.* **128**:514, 1914.

9. Bierende, F.: *Mitt. a. d. Grenzgeb. d. Med. u. Chir.* **32**:85, 1920.

resection for ulcer; (5) gastroenterostomy for ulcer; (6) Witzel's gastrostomy for carcinoma of the esophagus, and (7) resection of a large ovarian cyst. None of the patients had postoperative diarrhea. None survived for more than six days and 4 died within three days after the operation. In 5 of the 7 cases the diphtheritic lesion was restricted to the rectum; in the other 2 cases the colon was involved, diffusely in one, while in the other the lesion was segmentally restricted to the transverse colon. The most significant feature of this study lies in the detailed histologic observations and their correlation with the preoperative and postoperative status of the patient. Bierende's anatomic observations were essentially the same as ours; his analysis of these observations led him to consider the tissue changes as due to vaso-paralysis, which led to local circulatory disturbances. He believed that the latter, plus retention of feces, resulted in the terminal lesion.

Goldschmidt and Mülleder¹⁰ noted the occurrence of uncontrollable diarrhea in 30 patients in a series of 500 gastric operations of various types. They pointed out that the clinical symptoms were identical with those of dysentery but that culture of the stools did not yield pathogenic organisms. They presented detailed records of 3 cases and noted that in 7 others the patients recovered after presenting similar clinical features.

Their first case involved a 61 year old man with a pyloric obstruction of ten years' duration. This had finally become complete and had led to advanced cachexia and marasmus. Because of this, posterior gastroenterostomy was done. Six days later the abdominal wound was healed. On the eleventh day bloody diarrhea developed. Nine days later a gastrointestinal roentgenogram showed stenosis in the upper part of the jejunum. This was not found on laparotomy, which revealed a large amount of free fluid in the abdomen. The patient died two days later. Culture of the stool revealed no pathogenic organisms. Post-mortem examination revealed acute necrotizing colitis and ileitis.

The second case concerned a 55 year old man on whom subtotal gastrectomy was done for scirrhus carcinoma of the antrum. Twenty-four hours after the operation diarrhea developed, which persisted until the patient died, one week later. Culture of the stool gave a negative result. Autopsy showed widespread croupous, pseudodiphtheritic inflammation of the lower part of the ileum, as well as of the colon, with increasing intensity distally. The mesenteric vessels were patent. The peritoneal cavity contained about a liter of blood.

Their third case also involved a 55 year old man who had been subjected to subtotal gastrectomy. This was done for a duodenal ulcer and was made difficult by the presence of numerous adhesions. Six days after the operation diarrhea developed, which persisted until the man died, nine days later. Culture of the stool revealed no pathogenic organisms. Necropsy showed dehiscence of the duodenal stump with local peritonitis. In addition there was ulcerative colitis, with an ulcerative inflammatory lesion of the terminal ileum extending to the ileocecal valve.

10. Goldschmidt, W., and Mülleder, A.: *Mitt. a. d. Grenzgeb. d. Med. u. Chir.* 32:567, 1920.

In their discussion Goldschmidt and Mülleder mentioned the occurrence of similar necrotizing diphtheritic colitis following operation for tumor of the brain in 3 cases, as well as following traumatic fracture of the spinal column without operation in 1 case. Cultures of the stools in these 4 cases showed no dysentery organisms. In discussing the pathogenesis of these lesions they emphasized the alterations in intestinal flora consequent on gastric operations. This, they believed, when supplemented by peritoneal and retroperitoneal hematomas and vasomotor and vasosecretory disturbances led to the morphologic changes.

In a subsequent communication¹¹ they reported the occurrence of identical diphtheritic colitis in 5 patients with fracture of the spinal column. Of these, only 1 patient was operated on. Again bacteriologic examination of the stool revealed no pathogenic organisms. The presence of a hematoma of the mesentery in 1 patient led them to the belief that this predisposed to infection of the intestinal wall, which was aggravated by the vasoparalysis resulting from trauma to the spinal cord.

Within the past seven years a series of case reports have appeared in the French literature presenting observations which superficially resemble those already detailed.¹² In the main they present clinical observations without postmortem confirmation. In addition, it is to be noted that in these cases the intestinal lesion formed the primary condition and was present at the onset of the illness, so that these cases do not rightfully fall into the category with which we are concerned here.

CASE REPORTS

In a survey of the postmortem records of the Mount Sinai Hospital for the past ten years we were able to find 40 cases in which necropsy revealed pseudodiphtheritic ulceronecrotic enteritis, colitis or enterocolitis. In selecting these cases we did not include any in which the mesenteric artery or vein was thrombosed or in which there was present a diffuse vascular disease, such as periarteritis nodosa, malignant nephrosclerosis or chronic glomerulonephritis with uremia. The clinical data and pathologic material were complete in only 20 of these 40 cases. In the following case reports an attempt will be made to correlate the clinical features with the postmortem observations in an effort to indicate the pathogenesis of these lesions. The cases are typical of the entire group.

11. Goldschmidt, W., and Mülleder, A.: *Wien. klin. Wchnschr.* **35**:522, 1922.

12. Moulouguet, P.: *Bull. et mém. Soc. nat. de chir.* **57**:1504, 1931. Castellano, G.: *Policlinico (sez. chir.)* **41**:175, 1934. Schwartz, A.: *Mém. Acad. de chir.* **62**:1239, 1936. Grégoire, R.: *Bull. et mém. Soc. nat. de chir.* **60**:1394, 1934; **61**:634, 1935. de Fourmestreaux, J.: *Bruxelles-méd.* **15**:487, 1935. Grégoire, R., and Couvelaire, R.: *Apoplexies viscérales séreuses et hémorragiques (infarctus viscéraux)*, Paris, Masson & Cie, 1937.

CASE 1.—A 50 year old woman was admitted to the surgical service of Dr. A. A. Berg with a four month history of symptoms typical of ulcer. These had become so severe that operative intervention was decided on. A series of gastrointestinal roentgenograms showed the presence of a penetrating duodenal ulcer. A test meal showed free hydrochloric acid 48 and total acid 68. Hoffmeister's partial gastrectomy was done. The ulcer was embedded in the head of the pancreas but was dissected free and removed. On the night of the operation the patient was warm, the pulse rate was 128 beats per minute, and the blood pressure 140 systolic and 90 diastolic. Preoperatively the blood pressure was 120 systolic and 60 diastolic. Twelve hours after operation the patient began to vomit and was found bathed in sweat, with a pulse rate of 148, the pulse of poor quality. The blood pressure at this time was 70 systolic and 50 diastolic. She was given a transfusion of blood and continuous venoclysis of physiologic solution of sodium chloride, following which her condition showed distinct improvement, although she still complained of thirst. The pulse continued to be of poor quality, and the rate ranged between 140 and 150. At this time, three days after operation, the blood pressure was 125 systolic and 90 diastolic. On the next day the temperature rose to 104 F.; the pulse rate was 150 at the cardiac apex but was imperceptible at the wrist. The blood pressure dropped to 80 systolic and 60 diastolic, and the hands and feet were cold and clammy. The patient's face was pale and pinched, and she looked gravely ill. She was given 600 cc. of 10 per cent dextrose intravenously, after which the blood pressure rose to 124 systolic and 70 diastolic, and the pulse rate improved. Despite this, she rapidly became worse and died on the fourth day after operation. She had received several parenteral injections of digitan and caffeine and had had several "stimulating" enemas.

Postmortem Observations.—The peritoneal cavity contained about 200 cc. of bloody fluid. There was no evidence of generalized peritonitis. However, the duodenal stump was surrounded by an abscess cavity, which showed evidence of fat necrosis and had extended retroperitoneally to involve the right adrenal gland. The suture lines were intact. Extending from the lower portion of the sigmoid and involving the entire rectum there was a superficial greenish sloughing ulcerative membrane involving the entire circumference of the intestine and studded here and there with small hemorrhages. Microscopic examination of the involved area showed necrosis of the mucosa of the rectum, which was, however, not complete, since there were focal areas in which the deeper layers were preserved. The mucosa also showed leukocytic infiltration and deposition of fibrin, as well as surface colonies of gram-positive cocci. The submucosa was distinctly widened by edema fluid and showed focal hemorrhages. The submucosal vessels in the involved areas were dilated. The vessels outside the involved zones showed no significant changes. A lymph follicle located directly beneath the involved mucosa appeared to be without significant changes.

CASE 2.—This was the first admission to the surgical service of Dr. Richard Lewisohn of a 4½ year old child in whom abdominal pain, repeated vomiting and fever had developed about thirty-six hours previously. Examination revealed rigidity of the abdomen with tenderness and rebound tenderness. There was bilateral rectal tenderness with fulness on the right. Laparotomy disclosed a perforated appendix with generalized peritonitis. The appendix was removed. Fascial necrosis developed, which required removal of the sutures three days after the operation. At this time the general condition of the child was considered good. On the next day diarrhea developed which was attributed to the pelvic collection of pus. The child was febrile, the temperature reaching 105 F. On the

seventh day after the operation feces were noted in the wound and the abdomen became distended. Continuous venoclysis was instituted with physiologic solution of sodium chloride, and 0.5 cc. of a solution of posterior pituitary was injected intramuscularly, without relief. A Levine tube was passed, which drained off some bile-stained fluid tinged with blood. Ten days after the operation the child presented a typical peritonitic facies and a distended, silent abdomen. He was given a transfusion of 200 cc. of citrated blood. Despite this, he rapidly became worse and died in a semistuporous condition three days later.

Postmortem Observations.—The peritoneal cavity was the site of extensive though not generalized peritonitis, which caused the loops of intestines to be matted together. The entire intestinal tract was markedly dilated and showed the typical serosal reaction to peritonitis. The jejunum for the first 12 inches (30.5 cm.) beyond the ligament of Treitz was irregularly spotted with reddish purple hemorrhagic areas on its serosal surface. The mucosal surface corresponding to this discoloration was covered by a thick, coarsely granular and friable grayish yellow membrane, which extended over the rugae and troughs in an area 8 by 5 cm. The membrane was quite adherent and could be removed only with difficulty, leaving a rough surface. No other changes of importance were noted.

Microscopic examination of the involved jejunum revealed that the lesion did not involve the intestinal wall in a homogeneous fashion. There were areas of well preserved mucosa in which were well circumscribed foci of necrosis. The mucous membrane in these foci was replaced by a necrotic fibrinopurulent membrane, which extended into the submucosa. The most striking observation was the widening of the submucosa, due primarily to edema. The capillary vessels were widely dilated and blood filled, and in one area there was a focal submucosal hemorrhage. Some fibrin deposition was noted, and some infiltration by polymorphonuclear leukocytes. Several of the capillaries showed fibrin plugs. These submucosal changes extended beyond the area immediately adjacent to the foci of mucosal necrosis. The muscularis showed no significant changes. The serosa showed slight edema.

Microscopic examination of other portions of the small intestine showed no changes other than those due to the serosal reaction to peritonitis.

CASE 3.—A 23 year old plumber was admitted to the medical service of Dr. B. S. Oppenheimer with a three year history of hypertension, nocturia, polydypsia and incontinence. In the year preceding his admission he had had dyspnea on exertion, as well as edema of the ankles, and had noted an increase in the size of the abdomen and increasing weakness. He had also been troubled with frequent headaches and loss of libido. Examination showed an adult man with a buffalo type of obesity, involving the head, neck and trunk but not the limbs. He appeared plethoric and had typical pig eyes, as well as striae and kyphosis. His blood pressure was 170 systolic and 130 diastolic. There was cutis marmorata. The retinal arteries were narrowed, while the veins were engorged. The penis was small and underdeveloped. Both legs presented areas of chronic ulceration. The complete investigation led to the belief that the condition was typical of the Cushing syndrome. Exposure of the pituitary gland to the action of roentgen rays was ineffective. Bilateral perirenal air insufflation yielded no information of diagnostic value. Both adrenals were therefore explored by Dr. Edwin Beer, in sequence, without a tumor being disclosed. The postoperative course was perfectly smooth until the fifth day. At this time he became incontinent, and infection of a wound developed, culture of which disclosed *B. welchii*. On the seventh post-

operative day he went into "vasomotor collapse," the extremities becoming cold and clammy and the pulse rapid and small. He was given a venoclysis of 500 cc. of a 5 per cent solution of sodium chloride, as well as 12 minims (0.74 cc.) of epinephrine hydrochloride and 10 cc. of an extract of adrenal cortex (Eschatin) intravenously. Despite all these measures, he failed progressively and died twenty-four hours after the onset of the collapse.

Postmortem Observations.—There was no free fluid in the peritoneal cavity. The intestines were markedly distended and heavy with a fluid content. The serosal surface of the small intestines was dull gray with a pale blue tint. In some areas the serosal vessels were engorged. The serosal surface of the colon was a dull bluish gray. The colonic mucosa was dull and lusterless and was covered by a grayish green dirty membrane which could be removed only with difficulty. This lesion was present in the ascending colon and descending colon to the sigmoid and to a much lesser extent in the transverse colon, where it consisted of areas of engorgement.

Microscopic examination of the involved colon showed focal areas of extensive mucosal necrosis with deposition of fibrin and infiltration by leukocytes. Between the glands the capillary lumens were obliterated by hyaline masses. The submucosa was irregularly widened by edema, which was most pronounced just beneath the muscularis mucosa. The veins were markedly dilated and several contained nonadherent hyaline masses. The arterioles were filled with leukocytes and showed hyalinization of their walls. The muscular layers were without significant changes. The serosa showed venous dilatation.

CASE 4.—A 42 year old man was admitted to the surgical service of Dr. Harold Neuhoof with a two year history of recurrent fever, cough, expectoration of non-fetid sputum and hemoptyses. A roentgenogram of the chest revealed a breaking down in the upper lobe of the left lung. Bronchoscopic examination gave negative results. Exploratory thoracotomy was therefore done, and a superficial collection of pus was found in the lung. The postoperative course was smooth for the first nine days. At this time active oozing from the lung parenchyma was noted. This was treated by packing. On removal of the packing twenty-four hours later, bleeding recurred. The temperature rose to 104 F., and the hemoglobin content dropped from 80 to 60 per cent. He was therefore reoperated on eighteen days after the first operation, and a fat-muscle transplant was placed in the pulmonary cavity. About one month later bleeding recurred, and after six days of continued oozing, which was uncontrolled by packing, it was decided to perform lobectomy. The upper lobe of the left lung was therefore ablated. The blood pressure, which had been 114 systolic and 70 diastolic on admission, did not change significantly, but the pulse rate rose immediately after operation to 156 and became irregular and almost imperceptible. The patient became irrational, and it was found necessary to "cut down" in order to institute venoclysis. He continued thus for three days and then died.

Postmortem Observations.—An ulcer, 2 by 1 cm., was observed in the antrum of the stomach, saddling the lesser curvature and extending to the submucosa. Its edges were firm, raised and injected.

The serosal surface of the jejunum and ileum was smooth and glistening. In the distal 3 feet (90 cm.) of ileum there were scattered ulcers, 1 to 2 cm. in diameter, irregular in outline and shape and covered by a shaggy greenish exudate. Many of these ulcers were situated in Peyer's patches and presented congested, slightly raised, firm edges. The ileocecal valve was not involved. No lesions were noted in the colon or rectum.

Microscopic examination of the involved ileum showed widespread focal areas of necrosis of the mucosa, between which the mucosa was preserved to varying degrees. The involved mucosa was infiltrated by leukocytes and showed deposition of fibrin as well as fibroblasts and hyaline capillary thrombi. In these areas the submucosa was markedly widened by edema and showed a large number of dilated venules and capillaries. Some arterioles showed hyaline necrosis of their walls with definite hemorrhages in several foci. There were strikingly few leukocytes in these areas of edema, in some of which deposition of fibrin had occurred. The submucosal reaction was much less marked in areas where the mucosa appeared least involved but was nevertheless distinctly recognizable. The muscular layers and the serosa were without significant alterations.

CASE 5.—A 51 year old man was admitted to the surgical service of Dr. Richard Lewisohn with a ten year history of recurrent severe pain in the upper part of the abdomen, with nausea and vomiting. This had recurred ten weeks before admission and had been accompanied by sour eructations and hematemesis. A series of gastrointestinal roentgenograms revealed a deeply perforating lesion on the lesser curvature of the stomach. About three hours after the latter examination was completed, the patient suddenly experienced severe pain in the upper part of the abdomen, radiating to the left shoulder. Examination revealed the patient to be in shock and presenting marked abdominal rigidity and tenderness, most pronounced in the right upper quadrant. At operation, two and a half hours later, a perforated gastric ulcer was found and sutured. Postoperatively the patient fared poorly. He vomited repeatedly, and the abdomen was markedly distended. No relief was obtained by the use of a Levine tube or colonic irrigations. The temperature rose to 105.4 F. and the pulse rate to 120. The blood pressure showed no significant changes. Venoclysis was instituted, and epinephrine hydrochloride was given five times in doses of 5 minims. Despite this, the pulse became thready and rapid, a typical peritonitic facies developed, and the patient died five days after the operation, with a temperature of 106.4 F.

Postmortem Observations.—The peritoneal cavity showed about 1 ounce (30 cc.) of opaque yellow fluid. The intestines were markedly distended and filled with fluid. Their serosal surfaces were slightly dull, but deposition of fibrin was not noted. A Meckel's diverticulum was noted 3 feet (90 cm.) above the ileocecal valve. About a foot below the diverticulum there was an area about 3 inches (7.5 cm.) long where the ileal mucosal surface was covered by a diphtheritic membrane of greenish friable purulent material. The mesenteric border of the mucosa was not completely involved. About 1 foot (30.5 cm.) below this area an identical lesion involved about 1 foot of the intestinal wall. Just distal to this was still another patch of the same type, but smaller. The serosa in these regions was injected. The mesenteric vessels showed no gross changes. A few small red mesenteric nodes were seen. The colon and rectum showed no changes.

Microscopic examination of the involved ileum showed that the mucosal necrosis was focal in distribution and of variable severity. Where necrosis had occurred, the mucosa was replaced by a necrotic cellular débris, enmeshed in fibrin. In these areas the muscularis mucosa was involved in the process. The submucosa was markedly thickened by edema and some increase of connective tissue. The capillaries were widely dilated. There was no striking increase in cellular content in areas which were not severely involved in the necrosis. In the areas of lesser involvement the lesion did not extend to involve the muscular layers. In the more severely involved areas the process involved the muscularis down to the serosa, which showed some edema and cellular infiltration.

Histologic changes of the sort observed in these cases are certainly unusual and bear little resemblance to the usual inflammatory lesions found in the small or large intestines. We may perhaps best portray the lesions by a survey of the histologic changes in the entire group of cases which we have studied and thus reconstruct the various stages of their development.

Lesions in the earliest stages, found at a short distance from those in the more advanced stages or even in no relation to a lesion in an advanced stage, present as their most striking feature marked dilatation of the capillaries and venules, which may be so large as to distort the normal relationship of the tissues. This alteration occurs primarily in the submucosa but in a somewhat later stage is also found in the mucosa, where the sinus-like venous dilatation distorts the villi. This may occur in regions where the mucosa is apparently intact and therefore we feel that it represents an early stage of the process. Somewhat later there is noted, in addition to the aforementioned widening of the vessels, a widening of the stroma of the submucosa and mucosa by edema. The latter is strikingly poor in cellular content in its earlier phases but is frequently associated with focal hemorrhages in the immediate neighborhood of the distended vessels. These hemorrhages are found not only in the submucosa but in the villi as well. There is no apparent loss of continuity of the vessel walls, and one gains the impression that the extravasation is the result of diapedesis from a dilated capillary or venule in which stasis has occurred. In addition to these changes in the presence of well preserved mucosa, the progress of the lesion appears in alterations of the columnar cells which line the villi. The lining epithelium (at the onset usually only the portions at the tips of the villi) shows loss of nuclear staining and eventually the typical picture of necrosis (fig. 1 *A* and *B*). This, it must be emphasized, begins at the tips of the villi and is found in the presence of well preserved mucosa at the bases of the villi as well as between these. The necrotic areas of mucosa show focal desquamation. With the extension of the process, the necrosis involves the deeper layers of the stroma, beneath the mucosal cells, and may penetrate through the muscularis mucosa to involve the submucosa (fig. 2). With the progress of the lesion there is replacement of the mucosa by a membrane consisting of necrotic cellular débris enmeshed in fibrin. As the lesion progresses, leukocytes appear in the edematous stroma until eventually the appearance of typical purulent inflammation is created. It is impossible to evaluate the relative importance of infection from the intestinal lumen and of the presence of the necrotic mucosa in the causation of the latter reaction.

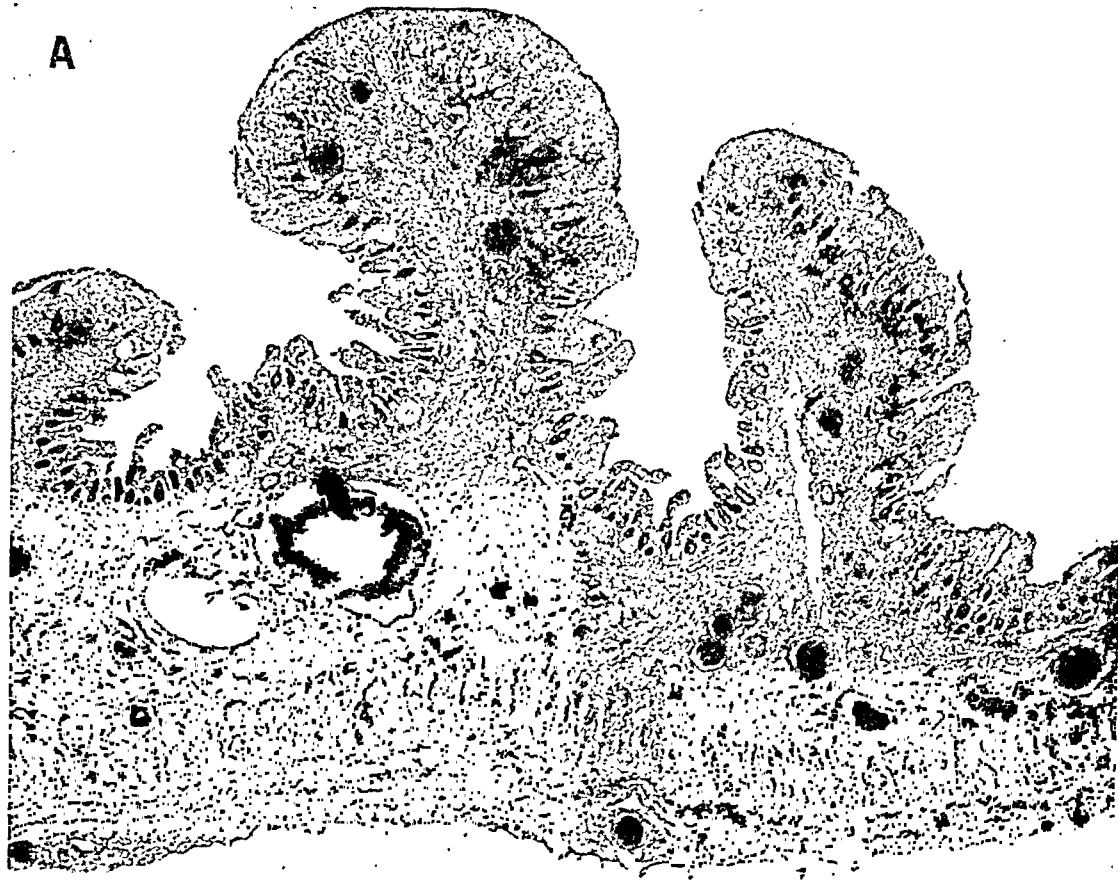


Fig. 1.—*A*, section of jejunum showing early necrosis of the tips of villi with well preserved mucosa at the bases. Widening of the submucosa, congestion and dilatation of the venules and capillaries, and interstitial edema can be observed.

B, the same section under higher power, to demonstrate the early necrosis of the tips of villi. Note the absence of primary cellular infiltration.

In the later stages of the process hyaline thrombi appear in many of the smaller vessels. In addition, in some instances, a striking inverse relationship is observed between the caliber of the arterioles and that of the capillaries and venules. In addition to the hyaline thrombi mentioned we have observed an occasional thrombus adherent to the wall of a vessel and containing leukocytes. One instance, at least, we observed, in which the inflamed submucosa showed the presence of fibroblasts.



/ Fig. 2.—Section of ileum showing focal mucosal necrosis and ulceration with submucosal reaction.

While in the majority of instances the process was restricted to the mucosa and submucosa, an occasional extension to the muscular layers was noted, so that it is apparently possible for the entire intestinal wall to be involved. Despite this, bacterial stains revealed organisms only on the mucosal surface.

The focal nature of the process is well shown by the fact that in a mucosal lesion that occurred in the duodenum Brunner's glands in direct contact with the involved mucosa showed no significant changes. In a similar lesion partial necrosis of Brunner's glands occurred. Similarly, in the colon it was observed that solitary lymph follicles which

were in direct contact with the necrotic mucosa were without significant changes. In other lesions the process involved the follicles also. These observations are of special significance when it is recalled that the solitary follicles, as well as Brunner's glands and the intestinal villi, receive their blood supply from totally separate arteriolar branches of the same submucosal artery.¹³

COMMENT

An investigation of the data on our material reveals that we have been dealing with a wide variety of clinical conditions. Among these are to be noted the following: gastric lesions, necessitating gastrectomy; perforation of a gastric ulcer; carcinoma of the lung with bleeding, requiring lobectomy; acute appendicitis with peritonitis; bilateral exploration of the adrenal glands, and, in addition, hepatic cirrhosis with hemorrhage, and extensive burns. Despite the wide variety of conditions, an analysis of the clinical data revealed that shock had been present in each instance and that it was usually present in direct relation to the few observed clinical signs of the intestinal lesion which we are discussing. This is true not only of our material but also of those cases reported in the literature the data on which are sufficiently complete to permit evaluation.¹⁴

The foregoing considerations led us to inquire into the possible processes whereby the vascular mechanisms involved in shock could be responsible for a lesion such as the one described. It will be recalled that this lesion is distinguished by the presence of dilated, usually blood filled venules and capillaries, early, relatively acellular submucosal and mucosal edema, pericapillary hemorrhages and finally focal mucosal necroses which by fusion, extension and secondary infection give rise to the more extensive pseudomembranous lesion.

At the present time it is widely accepted that the vascular mechanisms involved in shock serve the useful "purpose" of causing redistribution of blood so as to preserve the functions of organs absolutely necessary for life.¹⁵ Basically, this involves alteration of the volume capacity of the cardiovascular system in adaptation to a decrease in the volume of effectively circulating blood. The mechanisms effecting this adaptation constitute the characteristic vasomotor response seen in shock. That these mechanisms do not function uniformly throughout

13. Patzelt, V., in von Möllendorff, W.: *Handbuch der mikroskopischen Anatomie des Menschen*, Berlin, Julius Springer, 1936, vol. 3.

14. (a) Ewig, W., and Klotz, L.: *Deutsche Ztschr. f. Chir.* **235**:681, 1932. (b) Riedel.³ (c) Bierende.⁹ (d) Goldschmidt and Mülleder.¹⁰

15. Cannon, W. B.: *Physiol. Rev.* **9**:399, 1929.

the body was indicated in the work of Gesell,¹⁶ who showed that with decrease in blood volume of only 10 per cent there was simultaneous decrease of more than 60 per cent in the blood flow through the sub-maxillary gland. This occurred despite rise in blood pressure and was attributed to widespread vasoconstriction. The latter mechanism had been considered as the bodily response to shock as far back as 1879 by Mapother,¹⁷ who based his explanation entirely on clinical observations. In this he was supported by Malcolm,¹⁸ who noted the extreme pallor of the peritoneal surfaces in shock and made the observation that the arteries were empty while the venous trunks were full. The widespread increase in peripheral resistance caused by the vasoconstriction consequent on shock¹⁹ is not equally manifest in all organs. The work of Rein and his co-workers²⁰ and of Blalock and Levy²¹ showed that the vasoconstriction is most marked in the tissues which at the moment of application of the constricting stimulus are least active. This constriction may occur in the absence of rise in blood pressure and hence must be associated with compensatory vasodilatation elsewhere in the body. This vasodilatation is partially the result of local formation in actively functioning tissues of various metabolites that are effective in counteracting the vasoconstrictor stimulus.²² These observations serve partially to explain the occasional vasodilatation in response to epinephrine. Rein and Rössler^{20d} in one of their experiments were able to demonstrate that a loss of blood amounting to 2 per cent of the body weight in a dog resulted in a 20 per cent drop in blood pressure in eight minutes. Simultaneously the flow through the intestine decreased 70 per cent while that through the femoral artery dropped 62 per cent and that through the inferior vena cava 15 per cent. Ten minutes after cessation of the bleeding the blood pressure, after a preliminary further drop, rose to within 5 per cent of its previous value, the peripheral blood flow through the femoral artery rose to 30 per cent above its initial value, while the intestinal blood flow rose very much more slowly to a value which was 20 per cent below its original state. An interesting feature of this work lies in the demonstration that the vessels of

16. Gesell, R.: *Am. J. Physiol.* **47**:468, 1919.

17. Mapother, E. D.: *Brit. M. J.* **2**:1023, 1879.

18. Malcolm, J. D.: *Tr. M. Soc. London* **32**:274, 1909.

19. Erlanger, J.; Gesell, R., and Gasser, H. S.: *Am. J. Physiol.* **49**:90, 1919.

20. (a) Rein, H.: *Verhandl. d. deutsch. Gesellsch. f. Kreislaufforsch.* **10**:27, 1937. (b) Rein, H., and Mertens, O.: *Arch. f. d. ges. Physiol.* **237**:231, 1936. (c) Mertens, O.; Rein, H., and García Valdecasac, F.: *ibid.* **237**:454, 1936. (d) Rein, H., and Rössler, R.: *Ztschr. f. Biol.* **89**:237, 1929.

21. Blalock, A., and Levy, S. E.: *Am. J. Physiol.* **118**:734, 1937.

22. Gaddum, J. H.: *Gefässerweiternde Stoffe der Gewebe*, Leipzig, Georg Thieme, 1936.

the kidneys participate in the general vasoconstriction to a minimal degree.²³ It requires about one hundred times as much epinephrine to produce vasoconstriction in the kidneys as in the peripheral muscles. Of utmost importance in this connection is the fact that this vasoconstriction occurs as the first overt manifestation of the vasomotor response which is the body's reaction to the shocking stimulus, whether this is loss of blood,¹⁶ loss of body fluid²⁴ from the blood stream into the tissues or loss from the blood stream via the kidneys,²⁵ or painful stimuli.²⁶ In the final analysis we find that vasoconstriction may be considered compensatory for the marked discrepancy between the blood volume and the capacity of the cardiovascular system; or, as will be shown later, it may be one part of a vicious circle that causes loss of body fluid into the tissues. This concept reveals the fundamental harmony between the views of O'Shaughnessy^{26b, c} and Blalock.²⁴

The primary vasoconstrictive reaction does not and cannot persist indefinitely. It may be terminated by the passing of the shock stimulus. Under these circumstances the patient presents the picture of what may be called "compensated shock."^{14a} On the other hand, the vasoconstriction may disappear as a result of local tissue changes caused by the local ischemia which it creates²⁷ despite the persistence of the shock stimulus. There results an "outlying acidosis due to functional ischemia,"^{27a} which experimentally has been produced by injection of hypertonic dextrose or mechanical interference with the circulation. This has been known for a long time to be able to cause vasodilatation.²⁸ Aside from this, other metabolites capable of producing the same reaction are formed and undoubtedly participate in this process.²⁹

23. Hartmann, H.; Orskov, S. L., and Rein, H.: *Arch. f. d. ges. Physiol.* **238**: 239, 1936.

24. Blalock, A.: *Arch. Surg.* **22**:314, 598 and 610, 1931. Beard, J. W., and Blalock, A.: *ibid.* **22**:617, 1931.

25. Keith, N. M.: *Am. J. Physiol.* **68**:80, 1924.

26. (a) Freeman, N. E.; Shaw, J. L., and Snyder, J. C.: *J. Clin. Investigation* **15**:651, 1936. (b) O'Shaughnessy, L., and Slome, D.: *Brit. J. Surg.* **22**:589, 1935. (c) Slome, D., and O'Shaughnessy, L.: *ibid.* **25**:900, 1938. (d) Marquis, D. G., and Williams, D. J.: *Brain* **61**:203, 1938.

27. (a) Rous, P., and Drury, D. R.: *J. Exper. Med.* **49**:435, 1929. (b) Billings, F. T., and Maegraith, B. P.: *Quart. J. Exper. Physiol.* **27**:249, 1938. (c) Landis, E. M.: *Physiol. Rev.* **14**:404, 1934.

28. Gaskell, W. H.: *J. Physiol.* **3**:48, 1880. Bayliss, W. M.: *ibid.* **26**:xxxii, 1901. Ganter, G.: *Arch. f. exper. Path. u. Pharmakol.* **113**:66, 1926. Fleish, A., and Sibul, I.: *Arch. f. d. ges. Physiol.* **231**:787, 1933.

29. Zipf, K.: *Arch. f. exper. Path. u. Pharmakol.* **160**:579, 1931; **167**:60, 1932. Tannenberg, J., and Fischer-Wasels, B.: *Die lokalen Kreislaufstörungen*, in Bethe, A.; von Bergmann, G.; Embden, G., and Ellinger, A.: *Handbuch der normalen und pathologischen Physiologie*, Berlin, Julius Springer, 1927, vol. 7, pt. 2. Anrep, G. U.: *Studies on Vascular Regulation*, Stanford University, Calif., Stanford University Press, 1936. Gaddum.²² Billings and Maegraith.^{27b}

A further consequence of the vasoconstriction and the associated anoxemia of tissue is a drop in the gradient of pressure within the capillaries. This, together with the anoxemia, leads to an increase in the permeability of the capillary endothelium,^{27c} so that, whereas the latter formerly retained about 95 per cent of the total plasma protein, it loses its power to do this. As a result there occurs a transudation of the plasma protein into the tissue spaces. This is at first not accompanied by passage of the cellular components of the blood since in the earlier phases of this reaction, experiment has shown, the transudation of fluid is still directly proportional to the difference between the capillary pressure and the effective osmotic pressure of the plasma proteins. This observation apparently indicates that the capillary endothelium is still acting as a passive filter, though it is more permeable than usual.^{27c} Furthermore, this process is reversible if the lack of oxygen is of sufficiently brief duration. With persistence of the anoxemia and continuous loss of plasma protein into the tissue spaces a vicious circle starts, since reduction of the blood volume follows which in itself may cause vasoconstriction. In addition, the loss of blood plasma decreases the effective osmotic pressure of the intravascular contents and thus tends to increase the outflow of plasma. This concentration of the blood is invariably present in shock.³⁰

Further prolongation of the anoxemia of the capillary wall results in further changes in the capacity of the wall to retain the vessel contents. The cellular components and formed elements become increasingly concentrated, and the capillary wall itself becomes more "sticky" (Krogh, quoted by Landis ^{27c}). The morphologic component of these

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30. (a) Darrow, D. C., and Buckman, T. E.: *Am. J. Dis. Child.* **36**:248, 1928. (b) McIntosh, R.: Kajdi, L., and Meeker, D.: *J. Clin. Investigation* **9**:333, 1931. (c) Gibson, J. G., and Kopp, I.: *ibid.* **17**:219, 1938. (d) Rogers, F. T.: *Proc. Soc. Exper. Biol. & Med.* **38**:73, 1938. (e) Romberg, E.; Pässler, H.; Bruhns, C., and Müller, W.: *Deutsches Arch. f. klin. Med.* **64**:652, 1899. (f) Underhill, F. P.; Kapsinow, R., and Fisk, M. E.: *Am. J. Physiol.* **95**:302 and 325, 1930. Underhill, F. P., and Fisk, M. E.: *ibid.* **95**:330, 1930. (g) Davidson, E. C., and Matthew, C. W.: *Arch. Surg.* **15**:265, 1927. (h) Underhill, F. P.; Carrington, G. L.; Kapsinow, R., and Park, G. T.: *Arch. Int. Med.* **32**:31, 1923. (i) Harkins, H. N.: *Proc. Soc. Exper. Biol. & Med.* **32**:432, 1934. (j) Olivecrona, H.: *Acta chir. Scandinav.* **54**:559, 1922. (k) Lundgren, A. G. H.: *ibid.* (supp. 39) **77**:1, 1935. (l) Chang, H. C.; Harrop, G. A., and Schaub, B. M.: *J. Clin. Investigation* **5**:407, 1928. (m) Peters, J. P.; Kydd, D. M., and Eisenmann, A. J.: *ibid.* **12**:355, 1933. (n) Scott, H. G.: *Arch. Surg.* **36**:816, 1938. (o) Stewart, J. D., and Rourke, G. M.: *J. Clin. Investigation* **17**:413, 1938. (p) White, J. C.; Whitelaw, G. P.; Sweet, W. H., and Hurwitt, E. S.: *Ann. Surg.* **107**:287, 1938. (q) Gatch, W. D., and Little, W. D.: *J. A. M. A.* **83**:1075, 1924. (r) White, J. C.; Sweet, W. H., and Hurwitt, E. S.: *Ann. Surg.* **107**:438, 1938. (s) Riessinger, H., and Schneider, H.: *Deutsche Ztschr. f. Chir.* **217**:303, 1929. (t) Ewig and Klotz.^{14a}

functional changes is seen in the formation of capillary thrombi, observed in our material as well as in that studied by Bierende.⁹ A further consequence is seen in the appearance of hemorrhages which, in the absence of any morphologically demonstrable lesion of the vessels themselves, are attributed to diapedesis.

It is but a short step from the foregoing events to the appearance of the tissue necrosis which was seen to occur in our cases. That such a process may occur in shock has been known since 1906, when Elliott³¹ observed necrosis in the intestinal tract after bilateral adrenalectomy. Since then it has been repeatedly mentioned as an incidental observation at autopsy in experimental animals used in the study, directly or indirectly, of the phenomena of shock.³² The exquisitely focal nature of the lesions can be understood from the nature of the vascular mechanisms involved and from the anatomic distribution of the blood supply to the intestines as described in a foregoing paragraph. Thus the close juxtaposition of necrotic mucosa and well preserved Brunner's glands of submucosal lymphoid follicles is no surprise. The phenomena involved in the vasomotor reactions also make it readily understandable that the lesion which is created should take its origin within the wall of the intestine, i. e., in the submucosa, rather than on its surfaces.

We are not in a position to evaluate the importance of infection of the intestinal wall by the bacterial flora of the lumen. That infection occurs is evidenced by the purulent nature of the later stages of the lesion. That this is a late manifestation is indicated by its absence in the earliest phases of the lesion. In keeping with this is also the sudden rise in systemic temperature which so frequently terminated life in our material.

SUMMARY

A review of the literature of acute postoperative ulcerative and diphtheritic enteritis is presented. A survey of the postmortem records of the Mount Sinai Hospital for the last ten years revealed 40 cases in which necropsy showed this lesion. In the selection of cases we avoided any in which the mesenteric vessels were thrombosed or in which a diffuse vascular disease was present. Intestinal diseases of known cause,

31. Elliott, R. T.: *J. Physiol.* **49**:38, 1914. Elliott, R. T., and Tuckett, I.: *ibid.* **34**:332, 1906.

32. Freeman, N. E.; Shaffer, S. A.; Shecter, A. E., and Holling, H. E.: *J. Clin. Investigation* **17**:359, 1938. Unterberger, S.: *Arch. f. exper. Path. u. Pharmakol.* **2**:89, 1874. von Mering, J.: *ibid.* **13**:86, 1881. Saikowsky: *Virchows Arch. f. path. Anat.* **37**:346, 1866. Kebler, F.: *Arch. f. exper. Path. u. Pharmakol.* **9**:137, 1878. Elbe, D.: *Virchows Arch. f. path. Anat.* **182**:445, 1905. MacNider, W. de B.: *J. Exper. Med.* **27**:519, 1918. Heubner, W.: *Arch. f. exper. Path. u. Pharmakol.* **56**:370, 1907. Moon, V. H., and Kennedy, P. J.: *Arch. Path.* **14**:360, 1932. Keith.²⁵ Romberg and others.^{30e} Elliott.³¹

such as typhoid and dysentery, were likewise excluded. Five typical cases are reported in detail.

A survey of the histologic observations in our material permits us to reconstruct the pathogenesis of the lesion as follows: The earliest change consists in marked distention of the capillaries and venules, first in the submucosa and subsequently in the mucosa. This is followed by marked submucosal edema and occasional focal hemorrhage in the vicinity of the distended vessels (diapedesis). The arterioles frequently appear to be contracted. The next change consists in focal necrosis of the mucosa, frequently limited at first to the tips of the mucosal folds. With advance of the lesion the areas of mucosal necrosis spread and fuse. The necrosis extends through varying depths of the intestinal wall, although in most cases not beyond the submucosa. In the advanced stages, the necrosis is accompanied by an inflammatory cellular reaction, and hyaline thrombi are seen in many of the smaller vessels. The focal nature of the lesion is striking.

An analysis of the records showed that the lesion was present in a wide variety of clinical conditions. It appeared consequent to operative procedures in the abdomen, as well as after lobectomy, and it appeared in patients who had not undergone any operative procedures but were suffering from extensive burns and gastrointestinal hemorrhage. The one finding that was present in all the cases was shock.

We have reviewed the literature on the vasomotor mechanisms involved in shock and have discussed these in relation to the evolution of the lesion described and noted a similar lesion following experimental production of shock by a variety of methods.

CONCLUSION

From a study of 40 cases in which necropsy showed acute ulcerative or diphtheritic enteritis and from a review of similar cases in the literature we have concluded that the vasomotor mechanisms known to occur in shock are responsible for this lesion. Shock was present in all the cases studied, and a similar lesion has been observed following experimental production of shock.

CONGENITAL ABSENCE OF THE PENIS

GEORGE J. RUKSTINAT, M.D.

AND

ROBERT J. HASTERLIK, M.D.

CHICAGO

Congenital absence of the penis, according to Harris,¹ occurs in 1 of 30,000,000 persons. In the fifty years preceding 1898 it was encountered as follows: once each in England, France, Germany and Austria, and twice in the United States. In the last forty years 9 more instances were recorded. In at least 4 of these 15 instances the condition might be classified as hermaphroditism or scrotal penis. Excluding cases of epispadias, hermaphroditism and scrotal and perineal penis, one finds only 10 incontrovertible reports of congenital absence of the penis.

Steckmetz² published his findings in a 3 month old child whose penile site on the mons veneris was occupied by a furrowed elevation, 10 by 5 mm. In the subcutaneous tissue of the scrotum was a smegma-covered glans penis with a well formed foreskin. The shaft was rudimentary. A perineal midline structure was 10 mm. high and 3 mm. wide and resembled a rooster's comb. It was highest close to the anus but not connected with it. The rectum contained a small nubbin close to the sphincter on the anterior wall, into which the urethra opened. Göschler's³ patient, a 27 year old man, had orchitis but lacked a penis. On the perineum was a warty mass, 3.8 cm. long and 1.8 cm. wide and high. This contained erectile tissue, which had been stimulated first when the patient rode bareback. The man subsequently produced friction on this tissue with a stick or the edge of a chair to obtain sexual satisfaction. The urethra emptied into the rectum and was 3.8 cm. long. In Räuber's⁴ case approximation of the rectum and urethra was noted; part of the penis existed ectopically. His patient, a man 38 years old, lacked a penis in the usual position and passed urine through the rectum. Marked irritation from the urine had been noted for about twenty years. This was so severe at times that the man applied

From the Norman Bridge Pathological Laboratory, Rush Medical College of the University of Chicago.

1. Harris, R. P.: Philadelphia M. J. **1**:71, 1898.

2. Steckmetz, F.: Beitr. z. klin. Chir. **17**:398, 1896.

3. Göschler: Vrtljschr. f. d. prakt. Heilk. **63**:89, 1859.

4. Räuber: Virchows Arch. f. path. Anat. **121**:604, 1890.

hot poultices for relief and on occasion sat on ice. The penile elements present were incorporated in the anterior wall of the rectum. Passion manifested itself in a tickling sensation in the rectal wall and was accompanied by a seminal discharge into the bowel.

The irritating effect of urine on the rectum and probable ascending urinary infections were major complications in 2 patients: Räuber's⁴ and Mathews'.⁵ The latter was consulted by a 30 year old man who had no penis but had a well developed scrotum and large testes. The urethral opening was 2.5 cm. cephalad to the anal sphincter, on the anterior rectal wall. The rectal mucosa was ulcerated extensively and beset with hemorrhoids. Defecation and urination were painful, although some relief was obtained after massage of the hemorrhoids. The irritant effect of urine on the rectum of an adult contrasts with its innocuous effect in a child. Nélaton⁶ and Collier⁷ reported congenital absence of the penis with a urethral opening in the rectum in infants. The bowel movements of these patients were more liquid than normal but apparently the bowel wall was unaltered. Both adults who complained of rectal pain had fever, chills and sweats at various times and possibly suffered from cystitis or ascending urinary infection. Mathews' patient died of renal disease, supposedly uremia, about six months after the massage of hemorrhoids, mentioned. The 13 year old boy whose case was reported by Drury and Schwarzell⁸ has a urethral opening similar to that of the men discussed but has escaped rectal difficulties.

In the foregoing review the penile anomalies were grouped so that one might trace the stages of posterior urethral recession. The various urethral openings were noted anteriorly as in epispadias, perineally, anally and rectally in progressive steps. The most marked stage of maldevelopment is complete closure of the large bowel distally, with or without the presence of an anus or a vesicorectal fistula. These complicated anomalies are compatible with intrauterine life only and have been reported in newborn infants by Magid,⁹ Priesel¹⁰ and Feller and Sternberg.¹¹ Outstanding characteristics of this group are: absence of the penis, prostate gland and urethra; retention of one or both testes in the abdomen; termination of the rectum in a fibrous strand; a connection between the large bowel and the bladder by way of a patent channel

5. Mathews, J. M.: *Am. Practitioner & News* **17**:27, 1894.

6. Nélaton, A.: *Gaz. d. hôp.* **27**:45, 1854.

7. Collier, J., cited by Harris.¹

8. Drury, R. B., and Schwarzell, H. H.: *Arch. Surg.* **30**:236, 1938.

9. Magid, M.: *Monatschr. f. Geburtsh. u. Gynäk.* **83**:63, 1929.

10. Priesel, A., in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1931, vol. 6, pt. 3.

11. Feller, A., and Sternberg, H.: *Ztschr. f. Anat. u. Entwicklungsgesch.* **108**: 282, 1938.

or a fibrous union; ureteral stenosis or cystic distal termination; cystic changes of the kidneys, and bony deformities, notably a narrow pelvis and vertebral imperfections. This group, though small, has been helpful from an embryologic and physiologic standpoint. It had been assumed formerly that urine from the fetus contributed to the liquor amnii. Monstrosities lacking a urethral exit could not furnish urine to the liquor, yet in all instances the amounts of liquor bathing the child were normal. The bony deformities found in these bodies represent a stage only slightly above that of symphodia or of sirenoid monsters.

The following report of a case illustrates the several genitourinary anomalies and associated skeletal defects.

REPORT OF A CASE

A prematurely born white boy, 41 cm. long and weighing 2,020 Gm., was born by single footling breech presentation to a 19 year old mother after an uneventful forty hour labor. The baby did not cry or breathe and showed evidences of asphyxia. There was complete absence of the penis and anus externally (fig. 1), their normal sites being marked by smooth areas similar to the contiguous skin. The scrotum was 2 cm. in diameter and located in the normal position; its integument was wrinkled and pink-gray. No raphe was visible on the scrotum or on the perineum (fig. 1). The left foot was rotated and deformed so that its sole faced the median sagittal plane of the body. The right foot was normal. The toe nails reached to within 2 mm. of the ends of the great toes. No changes from the normal were observed in the skin, which was covered with fine lanugo hair.

The internal organs after fixation in a 4 per cent solution of formaldehyde disclosed the bladder, 1 by 3 by 1 cm.; in its wall, which was thickened to 4 mm. at its anterior superior border in the midline, was a nubbin of tissue, 12 by 5 mm., projecting superiorly from the wall, which represented a blunt closed urachus. At the right inferior posterior portion of the bladder externally, the distal end of a hugely dilated colon joined the wall of the bladder (fig. 3). Inferior and posterior to this anastomosis, the right ureter entered the bladder. At the corresponding point on the left side the left ureter made its entry (fig. 4). There was no urethral orifice in the bladder.

The testes with their epididymes measured 1.4 by 1.4 by 0.4 cm. and appeared grossly normal. The left was in the scrotum; the right lay on the left psoas at its midportion. The ductus deferens of each side became lost in a mass of fibrous and areolar tissue near the site of entrance of the ureters into the bladder. No prostate gland or seminal vesicles were found.

The right kidney was 8 by 10 by 7 mm.; its surface was knobby and cystic. The right ureter pursued a very tortuous course to its junction with the bladder. It measured up to 3 mm. in diameter externally, its wall was thickened markedly up to 1 mm., and in most places the lumen was of pinpoint size. The left kidney was 15 by 12 by 10 mm. Its surface was deformed markedly by cysts up to 6 mm. in diameter. The ureter 4 mm. distal to the ureteropelvic junction became a thin fibrous strand, 0.5 mm. in diameter, extending 8 mm., and again widened out into a more normal-appearing ureter, 2.5 mm. in external diameter.

The normal-appearing cecum and appendix lay in the right lower quadrant of the abdomen, as did the distal end of the gastrointestinal tract. The large bowel

measured 35 cm. from the cecum to its distal termination in the bladder. Immediately distal to the cecum the colon was 15 mm. in circumference, and the folds of the mucosa were very prominent. The colon 15 cm. distal to the cecum began progressively to balloon into a thin-walled structure, 55 mm. in circumference, with a wall 0.5 mm. thick. The mucosal surface here was smooth and without folds. Meconium was present in the distal 15 cm. of bowel. At its junction with the bladder the colon tapered abruptly to a point. A flap of the mucosa of the



Fig. 1.—*A*, posterior view illustrating the absence of the anus and of the scrotal raphe clubfoot on the left. *B*, anterior view demonstrating the absence of the penis and of the scrotal raphe.

bladder formed a valvelike structure which partially covered the opening of the colon into the bladder. This communication between the bladder and the colon was patent.

Two umbilical arteries and one umbilical vein were present. The left umbilical artery pursued a very tortuous course about 2 cm. before entering the umbilical cord.

The spinal column showed no obvious gross defects other than a deviation of the last few coccygeal segments toward the left. Roentgen examination of the spine revealed displacement of several ossification centers of the transverse processes of the fifth lumbar and first three sacral vertebrae.

The ilia showed no significant changes; the acetabulums appeared normal.

The inferior rami of the ischia were displaced medially and fused in the midline below the true symphysis pubis along the greater part of their course, forming what appeared to be a greatly elongated symphysis pubis, 28 mm. long. At a 50

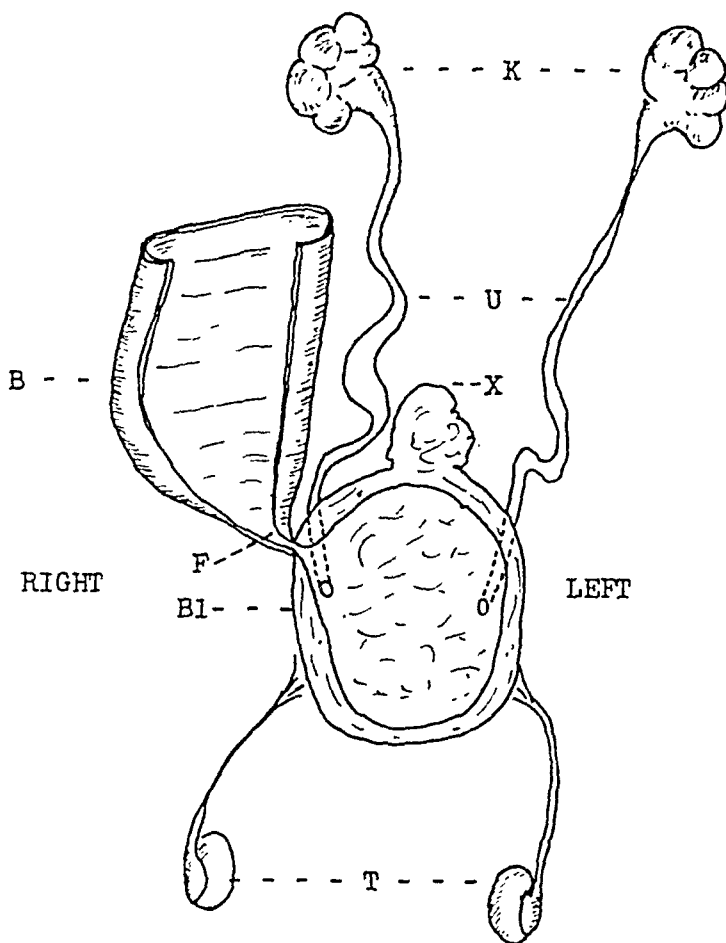


Fig. 2.—Schematic representation of anatomic relation. *B* indicates the bowel; *Bl*, the bladder; *T*, the testes; *U*, the ureters; *K*, the kidneys; *X*, the urachus, and *F*, the communications between the bowel and the bladder.

degree angle, where the ischia separated, were the ischial tuberosities, which at their greatest point were separated by a distance of only 12 mm. The distance between the ischial spines was 12 mm. also. From the tip of the coccyx to the point of bifurcation of the medianly fused inferior rami of the ischia, the anterior-posterior diameter of the outlet of this pelvis was 10 mm.

There was no asymmetry of the brain. No changes were observed in the hypophysis or in the large cranial venous sinuses. The cranial fossae were symmetric. The tongue, pharynx, larynx and roof of the mouth were normal.

Histologic Observations.—In the paraffin sections the right kidney, fixed in a 4 per cent solution of formaldehyde and stained with hematoxylin and eosin, hugely dilated veins occupied about 20 per cent of the section, had remarkably thin walls and were associated with distended tortuous capillaries. At least one third of the section consisted of old connective tissue, partly hyalinized and sparsely sprinkled with small lymphocytes. Localized clumps of 50 to 100 of these cells were scattered sparsely throughout the section. The arteries had thick walls (fig. 5), some of which were twice as thick as their lumen. At least half of this increased

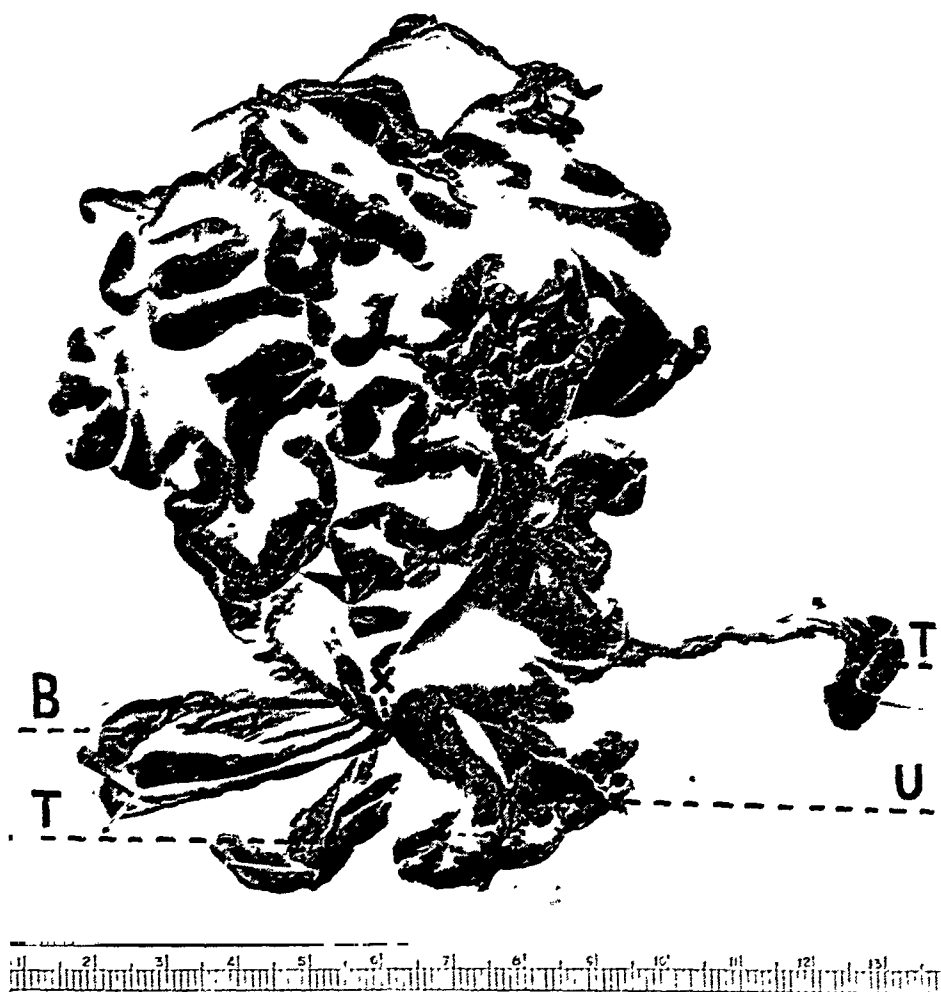


Fig. 3.—Anterior view of the pelvic and abdominal contents, illustrating at *B* the dilated part of the large bowel, at *U* the urinary bladder, at *T* the testes and at *X* the communication of bowel and bladder.

thickness was due to edema. Most of it was in the media, with the adventitia also involved. Collecting tubules occurred singly or in groups up to seven and were lined by high cuboidal epithelium. The cysts were irregular, lined by a compressed single layer of epithelial cells. Their bulk occupied about 30 per cent of the section; all the cysts were empty. No structures resembled glomeruli, but occasional partially hyalinized bodies suggested degenerated and organized glomeruli as in advanced nephrosclerosis.

In the left kidney glomeruli occurred, 5 to 8 per square millimeter. They were most numerous in two sharply circumscribed regions, each about 3.5 by 3 mm. Closely packed masses of epithelial cells were seen, concentrically layered or in a heterogeneous arrangement.

In both kidneys dilated tubules, especially outside of the fibrous snared-off regions, contained amorphous debris, in which were green globules resembling bile. At least three fourths of the cells within these tubules were lymphocytes. Only rare polymorphonuclear leukocytes and other inflammatory cell types occurred. In several regions, green globules with tan debris were observed in the walls

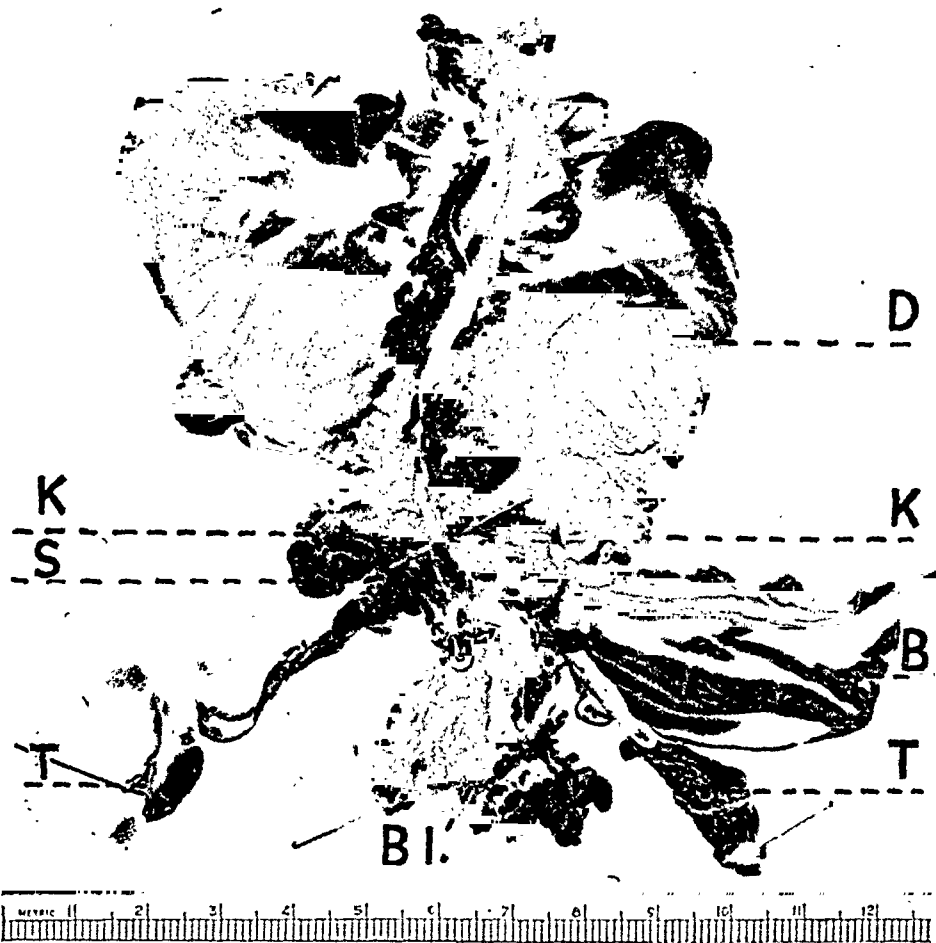


Fig. 4.—Posterior view of a dissection illustrating at *D* the diaphragm, at *U* the ureters, at *S* the ureteral stricture on the left, at *K* the kidneys, at *B* the dilated bowel, at *Bl* the urinary bladder and at *T* the testes.

of the blood vessels and in the connective tissue outside the tubules. A typical region showing the latter type of accumulation had a central area of necrosis, then a zone of sparsely applied fibroblasts and at the periphery collections of lymphocytes with fibroblasts.

Each cross section of testicle consisted about one-half of red blood cells free in the interstitial tissue. There was blood free in the tunica albuginea. In the epididymis there were lymphocytes in the loose connective tissue between the tubules, in irregular rows. They comprised about 10 per cent of the cells seen. There was extensive desquamation of the cells lining the tubules.

A section of urinary bladder close to the left ureteral orifice had a heavy muscular wall. The lining of the bladder was vacuolated extensively and consisted of four to five rows of transitional epithelium. In the first part of the left ureter the cell layers numbered up to eight. Only occasional scattered lymphocytes were observed in the submucosa of the bladder. The wall of the right vas deferens was twice the normal thickness and edematous. There were numerous lymphocytes, especially in the zone adjacent to the wall of the bladder.

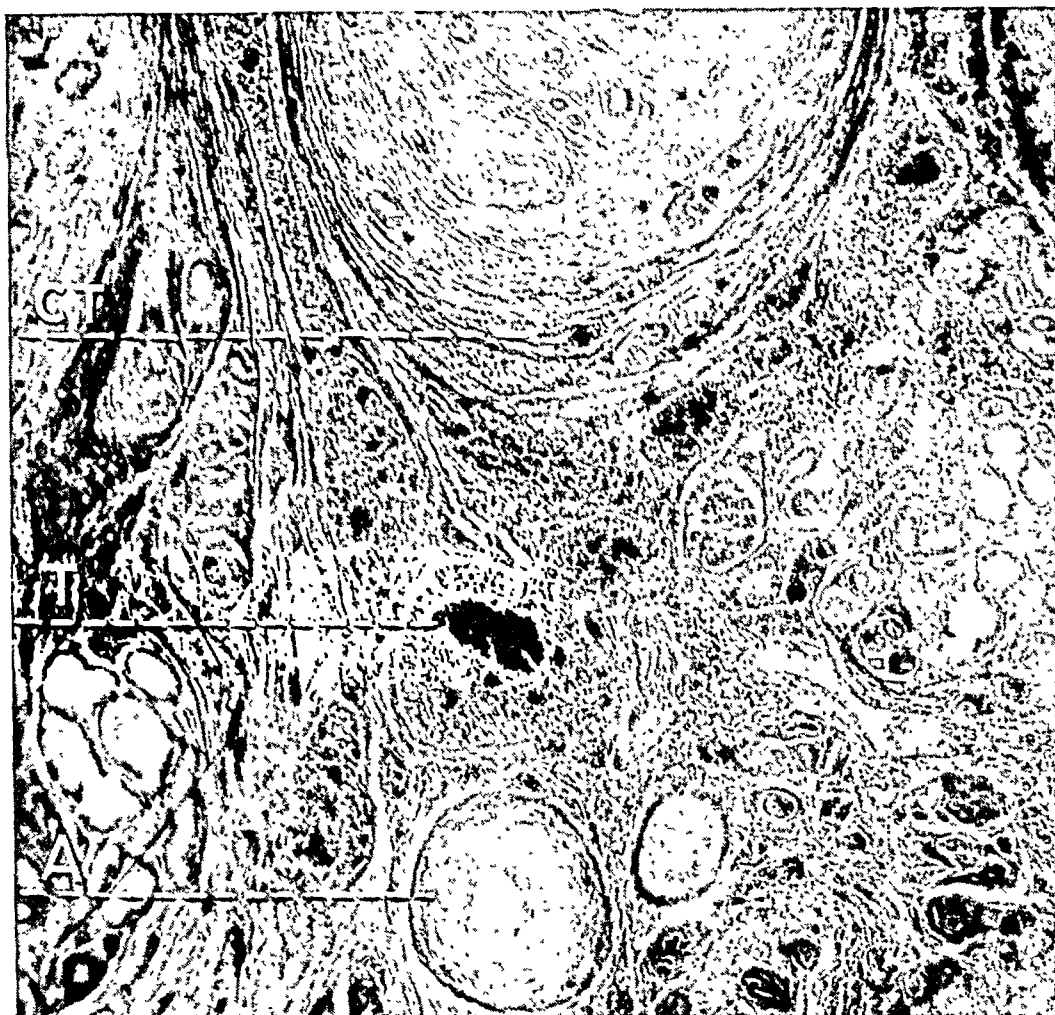


Fig. 5.—Low power photomicrograph of a section of the left kidney, illustrating at *A* a thickened artery, at *T* dilated tubules filled with debris, bile and lymphocytes and at *CT* the connective tissue bands enclosing hyalinized renal glomeruli and tubules.

A section of the fibrous mass in the region of the urachus was bladder with a wall as muscular as in the other sections and with an unaltered lining. Green globules, like those in smears of fresh meconium and in the dilated debris-filled tubules of the kidney, were seen in an unstained section of the kidney.

Sections of other internal organs revealed no pathologic changes pertinent to this problem.

COMMENT

An analysis of the conditions encountered in the present report corroborates Hinman's¹² opinion: "Malformations of the penis usually are associated with other anomalies, very often of the urethra. They are caused by maldevelopment of the phallus and genital tubercle, together with those portions of the urogenital sinus concerned in the formation of the external genitalia and urethra." The complete absence of the anus and rectum, however, further complicates the embryologic distortions. The absence of a perineal and scrotal raphe suggests a midline defect in early embryonic development. To produce the picture presented here, the cloaca must have failed to form the distal part of the bowel and contributed only the urinary bladder, omitting the proximal part of the urethra. The urogenital sinus, which normally contributes the rest of the urethra, failed to function, as did the genital tubercle, which should have differentiated into the penis and the genital folds which ordinarily form the prepuce and integument of the penis. The lateral genital swellings united to form the scrotum, which lacked only a raphe.

The acetabulums were deep and thin; they appeared disproportionately large in the narrow pelvis. The fused ischial rami and diminutive pelvic outlet, coupled with the displaced ossification centers of the distal vertebrae, suggested a relationship between the abnormality being reported and sirenoid monsters. The lower extremities, with the exception of the left clubfoot, were well developed.

The condition of the kidneys was especially interesting. Their alterations were vastly different from the hydronephrosis which usually results from obstruction to urinary outflow. The changes suggested marked chronic inflammation coupled with advanced benign nephrosclerosis. The inflammatory processes resulted in the production of broad constricting and intercepting connective tissue bands. These snared-off groups of tubules were dilated to produce cysts of various sizes and in general distorted the renal architecture. The tubules in places contained debris mixed with green globular material resembling bile. The most plausible explanation of the renal and ureteral changes is that meconium entered the bladder through the tract leading from the bowel. Mixed with urine, it easily passed to the kidneys in retrograde fashion, as the ureters were dilated distally. The bile then acted as an irritant in the tubules and induced an aseptic inflammation which produced the bizarre distortions noted.

12. Hinman, F.: *Principle and Practice of Urology*, Philadelphia, W. B. Saunders Company, 1935.

SUMMARY

A case of congenital absence of the penis, anus, prostate gland and urethra is reported in a premature infant who had a fistulous tract connecting his atretic large bowel and bladder. The marked inflammatory changes observed in the kidneys are attributed to the irritant effect of bile which entered the urinary system in meconium. The marked pelvic contraction and spinal defects suggest that this body possessed some of the characteristics of sirenoid monsters.

CHRONIC HYPOGLYCEMIA

REPORT OF TWO CASES WITH ISLET ADENOMA AND CHANGES IN THE HYPOPHYSIS

NATHAN B. FRIEDMAN, M.D.

NEW YORK

The syndrome of chronic hypoglycemia associated with islet tumors of the pancreas has become a well recognized clinicopathologic entity because of the striking correlation between the clinical, pathologic and physiologic findings. The review published by Whipple and Frantz¹ summarizes the observations in the earlier cases, and many further reports² have appeared, but there are still relatively few containing the results of complete autopsies.

I offer such a study of 2 patients with hypoglycemia who presented several interesting features and clinical problems. Islet adenoma, with islet hyperplasia, marked changes in the pituitary glands and several other lesions of interest were revealed at necropsy. The possible relationship of some of these changes to the hypoglycemia as well as to the obesity which developed in these patients will be discussed.

From the Medical and Laboratory Divisions, Montefiore Hospital for Chronic Disease.

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2. (a) Berry, J. A.: *Brit. J. Surg.* **23**:51, 1935. (b) Frank, H.: *München. med. Wchnschr.* **8**:1829, 1935. (c) Henner, K.; Jirásek, A., and Postránecký, O.: *Časop. lék. česk.* **75**:177 and 218, 1936. (d) Liu, S. H.; Loucks, H. H.; Chou, S. K., and Chen, K. C.: *J. Clin. Investigation* **15**:249, 1936. (e) Harnapp, G. O.: *Deutsche med. Wchnschr.* **62**:840, 1936. (f) Long, C. F.; Sheplin, L., and Fishback, D. B.: *Am. J. Digest. Dis. & Nutrition* **3**:488, 1936. (g) Aitken, L. F.: *M. Clin. North America* **20**:393, 1936. (h) Womack, N. A., and Cole, W. H.: *Ann. Surg.* **105**:370, 1937. (i) Hermann, S. F., and Gius, J. A.: *J. A. M. A.* **108**:1402, 1937. (j) Carlson, L. A., and Rynearson, E. H.: *Proc. Staff Meet., Mayo Clin.* **12**:386, 1937. (k) Lukens, F. W., and Ravdin, I. S.: *Am. J. M. Sc.* **194**:92, 1937. (l) McCaughan, J. M., and Brown, G. O.: *Ann. Surg.* **105**:354, 1937. (m) Reiter, G.: *Klin. Wchnschr.* **16**:844, 1937. (n) Kalbfleisch, H. H.: *Frankfurt. Ztschr. f. Path.* **50**:462, 1937. (o) Cragg, R. W.; Power, M. H., and Lindem, M. C.: *Arch. Int. Med.* **60**:88, 1937. (p) White, B. V., Jr., and Gildea, E. F.: *New England J. Med.* **217**:307, 1937. (q) Ziskind, E.; Bayley, W., and Mauer, E. F.: *Arch. Int. Med.* **60**:753, 1937. (r) Ziskind, E., and Bayley, W. A.: *J. Lab. & Clin. Med.* **23**:231, 1937. (s) Fraser, R.; MacLay, W. S., and Mann, S. A.: *Quart. J. Med.* **7**:114, 1938. (t) Malamud, N., and Grosh, L. C., Jr.: *Arch. Int. Med.* **71**:579, 1938.

REPORT OF CASES

CASE 1.—Summary.—This patient had suffered from seizures for about two years before the diagnosis of chronic hypoglycemia was made at another institution. He was studied there for one and a half years, and at laparotomy for suspected pancreatic adenoma no tumor was found. He was transferred to this hospital and studied for two years before death occurred during a hypoglycemic attack. Necropsy revealed an islet adenoma of the pancreas and marked adenomatous hyperplasia of the chromophile cells of the pituitary.

History.—A 50 year old Jewish man was admitted to the Montefiore Hospital in June 1935. In October 1933 he had been studied elsewhere for suspected tumor of the brain. At that time he had complained of seizures varying from dizzy spells to episodes of peculiar behavior or even unconsciousness, of which he had been aware for about two years. He was rehospitalized at a second institution in February 1934, after considerable progression of his symptoms. The association of his seizures with low levels of the blood sugar and the relief of symptoms on intravenous administration of dextrose were observed, and the diagnosis of chronic hypoglycemia was established.³ The presence of extrapyramidal signs, such as parkinsonian facies and gait, loss of associated movements, tremors and slurred, syllabic speech, were considered significant. At laparotomy for suspected islet adenoma of the pancreas, no tumor was found, and biopsies of the liver and pancreas gave essentially negative results.

On examination in June 1935, the markedly obese patient was 160 cm. in height and weighed 92 Kg. An incisional ventral hernia was present. The blood pressure on numerous occasions ranged from 120 systolic and 80 diastolic to 170 systolic and 100 diastolic. The picture suggestive of parkinsonism described was present.

The urine and blood counts were essentially normal. The Wassermann reaction of the blood was negative. The blood sugar was 30 to 60 mg. in 100 cc. (fasting); lactic acid, 15.7 mg.; urea nitrogen, 5 to 12.1 mg.; uric acid, 3.3 mg.; creatinine, 1.8 mg.; cholesterol, 122.9 to 149 mg.; cholesterol esters, 78.8 to 112 mg.; serum albumin, 3.2 to 4.9 mg.; serum globulin, 2.1 to 3.7 mg.; calcium, 10.5 mg.; phosphorus, 3.4 mg.; chlorides, 566 to 604 mg.; carbon dioxide-combining power, 52 to 63 volumes per cent. Sugar tolerance tests showed a rise from low levels to about 150 mg. at one hour with a fall to low levels at two to three hours. Epinephrine (1 cc. of a 1:1,000 solution) caused a rise in blood sugar (60 to 90 mg.) and lactic acid (15.7 to 36.9 mg.) and a fall in phosphorus (3.7 to 2.5 mg.). Insulin caused no more marked fall in the blood sugar than was present spontaneously. It did, however, decrease somewhat the hyperglycemia following administration of dextrose. There was no creatinuria, and the urinary nitrogenous constituents were normal.

Roentgen examination showed a normal sella turcica. The basal metabolic rate (nonfasting) was plus 9 per cent and plus 3 per cent (average calories per hour, 77.8; normal for height and weight, 73.3).

Course.—The previous regimen of a high carbohydrate diet with added feedings was continued. When food was withheld, hypoglycemic symptoms appeared. In mild attacks restlessness, unresponsiveness, sluggishness or sometimes aggressiveness, with paranoid trends, were noted. In more severe attacks the patient became semistuporous, thrashing about with no definite convulsive pattern. During these attacks salivation and diaphoresis were marked, and a positive Babinski sign could

3. Blau, A.; Reider, N., and Bender, M. B.: *Ann. Int. Med.* **10**:910, 1936.

be obtained. The oral administration of sugar during a mild episode relieved the symptoms in about fifteen minutes, but more severe attacks necessitated administration of dextrose by stomach tube or by intravenous injection. The injection of as little as 1 Gm. of dextrose, with only minimal elevation of the blood sugar, was often striking in its almost immediate restoration of the patient.

During seizures there were no essential changes in the chemical constituents of the blood with the exception of dextrose. The respiratory and pulse rates and the blood pressure rose; extrasystoles or pulsus alternans was occasionally noted, and the skin was mottled and cyanotic. The blood volume (congo red) was unchanged, and there was moderate leukocytosis, but there was no other change in the blood picture.

After prolonged attacks there occasionally occurred spontaneous but temporary improvement with a rise in the blood sugar.

It was found necessary to give the patient a diet of between 50 and 75 Gm. each of protein and fat and of from 700 to 1,000 Gm. of carbohydrate, with frequent feedings, in order to maintain his blood sugar above shock levels. On this regimen his weight rose from 92 Kg. on admission to 130 Kg. at his death, two years later.

Thyroid extract was given on several occasions to the point of toxicity, with a basal metabolic rate of plus 44 per cent (117.9 calories per hour), a treatment which occasionally held his weight level for a few weeks at a time.

With ephedrine, as well as with thyroid extract, the blood sugar levels were slightly higher, but no significant decrease in carbohydrate intake was possible.

The patient complained of increasing weakness, which was not aided by benzedrine, ephedrine and prostigmine, and of difficulty in walking and speaking. He had considerable gastric distress accompanying feedings. Under full atropinization the blood sugar levels were generally lower, although there was considerable alleviation of the difficulty in speaking and walking.

In November 1936 irradiation of the pituitary was tried, the doses totaling 750 roentgens over about a nine day span. Severe exacerbation of the hypoglycemic state followed, so that on a carbohydrate intake on which he had been shock free for some weeks he had frequent severe seizures. The following figures for blood sugar reveal the marked alteration, which was temporary.

	Before Dextrose (30 Gm. Given Orally)	At Given Number of Minutes After Dextrose			
		30	60	90	120
Control	65	130	150	100	55
After irradiation	68	102	47	41	..

Beginning in March 1937, a higher protein diet was given, but the carbohydrate intake could not be reduced significantly. The blood urea nitrogen, however, which had been as low as 5 mg., rose to 12. Protein in the forms of casein (100 Gm.) and meat (400 Gm.) was also substituted for the added carbohydrate in two trials (Conn⁴). The blood sugar level, however, was not maintained, and the onset of hypoglycemic symptoms necessitated the use of carbohydrate.

During 1937 numerous small filiform papillomas appeared on his face, neck and chest. In August 1937 an acute respiratory infection developed, with a rise in temperature to 103 F. During this period it was found possible to cut down his carbohydrate intake considerably and still avoid seizures. This had been noted during a similar episode in December 1935.

On recovery from the infection he had many seizures, and although his carbohydrate intake was increased steadily, the attacks persisted. On the evening

4. Conn, J. W.: J. Clin. Investigation 15:673, 1936.

of his death he was seen to be restless, and later he lapsed into a typical seizure. On intravenous administration of dextrose he did not respond as usual but became markedly cyanotic and went into collapse, from which he died despite administration of epinephrine.

The conditions finally diagnosed clinically were: chronic hypoglycemia; obesity; encephalopathy of unknown origin; possible islet adenoma of the pancreas.

Pathologic Observations.—The anatomic diagnosis (Dr. David Perla) was: obesity; islet adenoma of the pancreas; basophilic and eosinophilic adenomatous hyperplasia of the anterior lobe of the pituitary; bilateral cortical adenomas of the adrenals; tubular adenoma of the kidney; polypoid adenomas of the ileum; papillomas of the skin; hyperplasia of the interstitial cells of the testis; hypertrophy of the prostate; hyperplasia of the spleen; chronic gastritis; marked congestion and cyanosis of the viscera; cardiac hypertrophy.



Fig. 1.—Gross appearance of the pancreatic adenoma in case 1.

The body showed extreme generalized obesity. A midline abdominal healed surgical scar, 30 cm. in length, was present. The pupils were dilated, and the face was markedly cyanotic. There were many pedunculated papillomas and fibromas over the face and chest.

All of the viscera showed extreme congestion and cyanosis. The heart weighed 500 Gm. The lungs showed moderate congestion and edema. The 2,500 Gm. liver was intensely congested and cyanotic. The 180 Gm. spleen was grayish red and diffuent. The kidneys together weighed 450 Gm. and were markedly congested. The adrenals together weighed 18.5 Gm., and both showed small cortical adenomas. The thyroid weighed 24 Gm. The seminal vesicles, prostate, bladder and testes appeared normal.

The pancreas weighed 160 Gm. and was very cyanotic and friable. In the tail was a firm nodule, 3 by 2.5 by 2 cm. (fig. 1). It was moderately well encapsulated and showed on section a grayish yellow surface with grayish streaking extending from the capsule.

The brain weighed 1,240 Gm. The right lateral ventricle was narrowed and distorted, and the posterior horn was slightly dilated.

The pituitary weighed 620 mg. On section there were numerous opaque nodules in the anterior lobe. The pineal gland was calcified.

Microscopic Observations.—The heart showed moderate hypertrophy of the muscle fibers. The lungs and liver showed extreme congestion. The kidneys were congested, and a single small tubular adenoma was noted. The adrenals were markedly congested and showed several lipoid-rich cortical nodules. The prostate showed slight adenomatous hyperplasia. The stomach showed thinning of the mucosa and extensive round cell and slight polymorphonuclear infiltration. The testis showed proliferation of the interstitial cells, particularly in one region, where the tubules were atrophic. Spermatogenesis was diminished. The thyroid, parathyroids, spleen and pineal gland showed no essential abnormalities.

The major lesions were in the pancreas and pituitary. The nodule in the pancreas was composed of tissue resembling that of the islets of Langerhans (fig. 2). It showed ribbons and cords of columnar and cuboidal cells, with a thin, delicate stroma of connective tissue and capillaries. In many areas the nuclei, which were oval and vesicular, were basally placed with respect to the capillaries. The fibrous stroma was increased in some portions, and there were isolated nests and clumps of cells. There was a definite fibrous capsule containing similar cell masses. The cytoplasm of the cells was acidophilic and granular.

After fixation in solution of formaldehyde U. S. P. a portion of the adenoma was refixed in a dilute Helly solution and stained with fuchsin orange by the method of Bayley.⁵ The cells uniformly showed reddish granules in the cytoplasm. These granules were not as brilliant red as those seen in the beta cells of a section of freshly fixed guinea pig pancreas used as a control. The remainder of the pancreas had undergone such extensive degeneration, presumably post mortem, that histologic studies were out of the question.

The pituitary was fixed in Orth's solution, was sectioned in seven sagittal planes and stained with hematoxylin and with Mallory's connective tissue stain for study (Dr. Charles Spark).

The anterior lobe showed striking hyperplasia of both basophilic and eosinophilic elements with reduction in the number of chromophobes.

The basophilic adenomatous hyperplasia was most marked. There were numerous poorly circumscribed nodules up to 1 mm. in diameter, composed of atypical basophilic cells. These were irregular in size and shape, with large vesicular nuclei, and there were bizarre giant forms and syncytial structures. Transitional forms and cells showing loss of granules were also present in abundance, but vacuoles were scanty and no "colloid" basophils (Crooke⁶) were seen.

Several larger, fairly well circumscribed nodules, up to 4 by 2 mm. in size, with definite compression of capillaries and of the surrounding tissue, were seen (fig. 3). These were composed chiefly of large, fully granulated basophils, although some cells showed pyknotic nuclei and agglutinated granules.

The acidophils were also increased in number, but most of them appeared as broad sheets of normal, fully granulated cells. There was a single 2 by 1 mm. nodule of transitional lightly granulated forms. Near the pars intermedia was an area of large, irregularly shaped cells, only moderately well granulated, but

5. Bayley, J. H.: J. Path. & Bact. **44**:272, 1937.

6. Crooke, A. C.: J. Path. & Bact. **41**:339, 1935.

with huge vesicular nuclei and very prominent nucleoli, resembling cells seen in malignant tumors.

Throughout the anterior lobe there was little follicle formation and no colloid retention. The connective tissue was not increased.

The region of the pars intermedia showed large cysts filled with colloid, and there was moderate infiltration of the pars nervosa with small, irregularly shaped, fairly ripe basophilic cells. However, the degree of infiltration was only a fraction

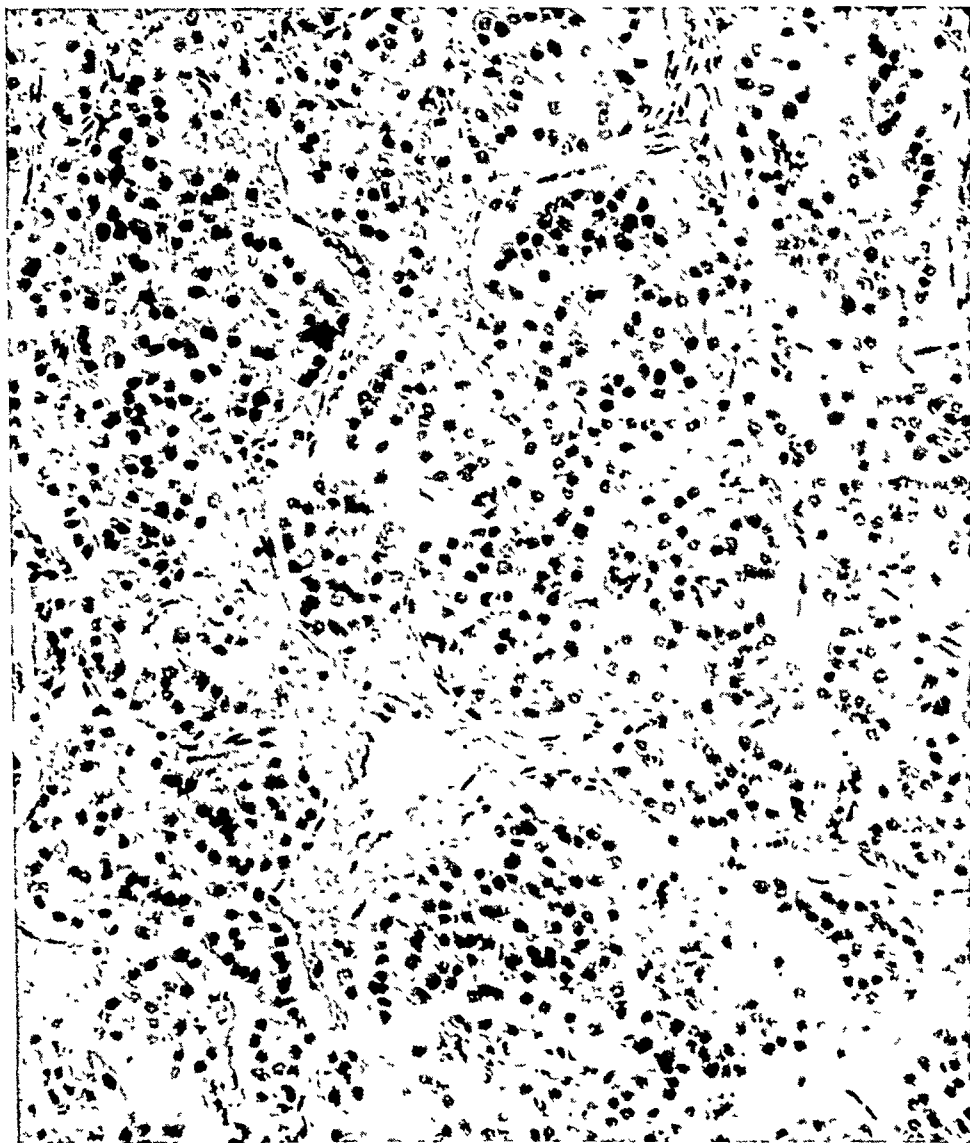


Fig. 2.—Microscopic appearance of the pancreatic adenoma in case 1.

of that seen in the second case. There was no increase in the pigment of the posterior lobe.

A study was made of the brain cortex, basal ganglions, mesencephalon, pons and medulla (Dr. Charles Davison). The vessels were congested, and there were scattered small hemorrhages (agonal). Studies of myelin sheaths showed no abnormalities. In all the regions examined, some of the nerve cells showed the changes of ischemia.

In addition to these changes, some of the cortical nerve cells showed the so-called water changes. In the caudate and putamen occasional nerve cells were disintegrated, and a few Alzheimer glia cells (types 1 and 2) were noted. There was marked calcification of some of the pallidal blood vessels. There was an occasional water cell in the thalamus.



Fig. 3.—A nodule in the pituitary in case 1.

The cells of the nucleus supraopticus, nucleus paraventricularis, nucleus reuniens, nucleus mamilloinfundibularis and nuclei tuberis showed poverty of the usual pigmentary deposits, and in the nucleus basalis such deposits were absent. There were a few vacuolated cells in one area of the nucleus paraventricularis.

CASE 2.—*Summary.*—The patient had suffered from seizures for three years, which were associated with a low level of the blood sugar and could be relieved by the administration of sugar. Focal neurologic signs were present. When

increased intracranial pressure became evident, study suggested a tumor of the left side of the brain. Craniotomy revealed a large subdural hematoma, which was removed, but the patient died eighteen days after the operation. Necropsy revealed an islet adenoma of the pancreas, marked hypertrophy and hyperplasia of some of the islets of the pancreas, marked adenomatous hyperplasia of the chromophile cells of the pituitary and a fungus infection of the esophagus.

History.—A 56 year old Jewish man was admitted to the Montefiore Hospital in January 1936. He gave a history of occasional attacks of dizziness over a period of three years, associated with stiffness and loss of control of the right arm and leg during seizures. In the year preceding admission he had seizures while asleep, during which he mumbled and shouted, thrashed about with his right arm and leg, became flushed and perspired, and from which he could not be aroused. The attacks increased in frequency and were relieved or prevented by the taking of food or orange juice. Increasing hunger was noted, and the patient had gained about 25 pounds (11.3 Kg.) in weight in six months. During one attack in September 1935 he had fallen down a flight of stairs.

Examination showed an obese man, weighing 74 Kg. and measuring 160 cm. in height. The blood pressure was 130 systolic and 90 diastolic. There were tortuous peripheral vessels and slight retinal arteriosclerosis. There was very slight right hemiparesis.

The urine and blood counts were normal. The Wassermann test of the blood was negative. The blood sugar was 45 to 59 mg. in 100 cc. (fasting); lactic acid, 16.3 mg.; urea nitrogen, 16.8 mg.; calcium, 12.2 mg.; phosphorus, 4.8 mg.; chlorides, 597 mg.; serum albumin, 4.7 mg.; serum globulin, 3.2 mg.; cholesterol, 122 mg. Sugar tolerance tests revealed a basal level of 50 mg. or lower and a peak of over 100 mg. at one hour and a fall to low levels at three hours. Epinephrine caused a rise in blood sugar (56 to 95 mg.) and lactic acid (16.3 to 40.5 mg.). Insulin did not recognizably accelerate the spontaneous fall in blood sugar but decreased the hyperglycemia induced by dextrose.

Shortly after admission the patient was seen in an attack, during which he became noisy and nonresponsive. He sweated, flushed and had occasional twitchings of the mouth and hands. Such seizures could be produced by fasting and were associated with low levels of the blood sugar. Dextrose given by mouth relieved attacks in from ten to fifteen minutes, and dextrose injected by vein was almost immediately active, even in small amounts (less than 5 Gm.).

The patient was placed on a diet high in carbohydrate (370 Gm.), with frequent added feedings to maintain the blood sugar above shock levels.

In April 1936 nausea and vomiting gradually developed, with frontoparietal headache on the left side.

The essential observations were those of right hemiparesis, which had not progressed from that noted on admission and which had been considered the residuum of an old unnoticed cerebral vascular accident. The fundi revealed hyperemic disks with blurred margins and engorged veins, although there was no measurable elevation.

Lumbar puncture disclosed a spinal fluid pressure of 260 mm. of water. The fluid was normal. The temperature at about this time had risen occasionally and irregularly to 102 F. without obvious cause. The patient had become partially disoriented, facetious and euphoric. Encephalography revealed the left lateral ventricle to be larger than the right and displaced beyond the midline. The right lateral ventricle was partly filled and was displaced to the right and downward. The third ventricle was dilated and displaced to the right.

On April 29 a left frontoparietal craniotomy was performed by Dr. Ira Cohen, the temperature having fallen to normal preoperatively. A huge subdural hematoma was disclosed extending over practically the whole left hemisphere and beneath the temporal and frontal poles. It was up to 3 cm. in thickness and was partially liquefied, containing 50 cc. of dark brown material. The entire hematoma, which was somewhat adherent to the dura, but practically not at all to the arachnoid, was removed in pieces.

Postoperatively there was no change in the hypoglycemic state, and the blood sugar was maintained above shock levels on the same regimen as was described.

At first the patient's general condition was good, but on the eleventh postoperative day his temperature began to rise, and he became semistuporous, with Cheyne-Stokes respiration, and had intractable hiccup, abdominal distention and incontinence. When a series of convulsions involving the left arm and the right leg appeared, a postoperative hematoma was suspected, but bilateral frontoparietal bore holes revealed none. On May 16, with a blood pressure of 80 systolic and 55 diastolic, pulmonary edema developed and the patient died, with a terminal temperature of 104 F. The blood sugar on the last two days had risen to 280 and 320 mg. on the forced carbohydrate regimen, but there was no acetonuria.

The conditions finally diagnosed clinically were: chronic hypoglycemia; obesity; status after craniotomy for subdural hematoma; possible islet adenoma of the pancreas.

Pathologic Observations.—The anatomic diagnosis (Dr. Henry Brody) was: obesity; hyperplasia of the islets of Langerhans with the formation of single large islet adenoma; basophilic and eosinophilic adenomatous hyperplasia of the anterior lobe and basophilic infiltration of the posterior lobe of the pituitary; craniotomy; extradural hematoma; bronchopneumonia of the left lower pulmonary lobe; fibromyoma of the esophagus; pseudomembranous (fungous) esophagitis.

The body showed marked generalized obesity, and there was a great abundance of fat in the usual depots. There was a partially healed semicircular scalp incision in the left frontoparietal region, with a loose underlying bone flap. There was a second short incision over the right frontoparietal area, with a small bone defect underneath.

The heart weighed 400 Gm., and there was moderate atherosclerosis of the coronary arteries. The lungs were congested, and the left lower lobe showed small patches of bronchopneumonia with fibrinous pleuritis. The esophagus was adherent to the adjacent structures and showed marked wrinkling and thickening of the brownish mucosa. At the junction of the esophagus with the stomach was a fibromyoma, 2 by 1 cm. The stomach was dilated. The liver (2,500 Gm.), the spleen (210 Gm.) and the kidneys (370 Gm. together) appeared normal.

The pancreas was normal in size and shape and showed a moderate interlobular infiltrate of fat. At the junction of the body and head, at the upper edge of the posterior surface, was a firm spherical 1 cm.-sized encapsulated nodule, fairly well demarcated from the surrounding pancreatic tissue. It cut with increased resistance, showing a chalky white surface with a few 1 mm.-sized purplish areas.

Under the left frontoparietal bone flap there was a fairly large organized extradural hematoma. The 1,700 Gm. brain was flattened in the region of the flap, was generally congested and was slightly indented over the right orbital convolutions. On section the left ventricle was constricted, while the third ventricle was distorted and shifted to the right.

The pituitary weighed 660 mg. On sagittal section opaque whitish tissue replaced most of the normally glistening and translucent posterior lobe. The anterior lobe showed numerous small whitish opaque nodules.

The pineal gland appeared normal. The thyroid, parathyroids and testes were not examined.

Microscopic Observations.—The liver showed a few small foci of fatty change and slight central congestion.

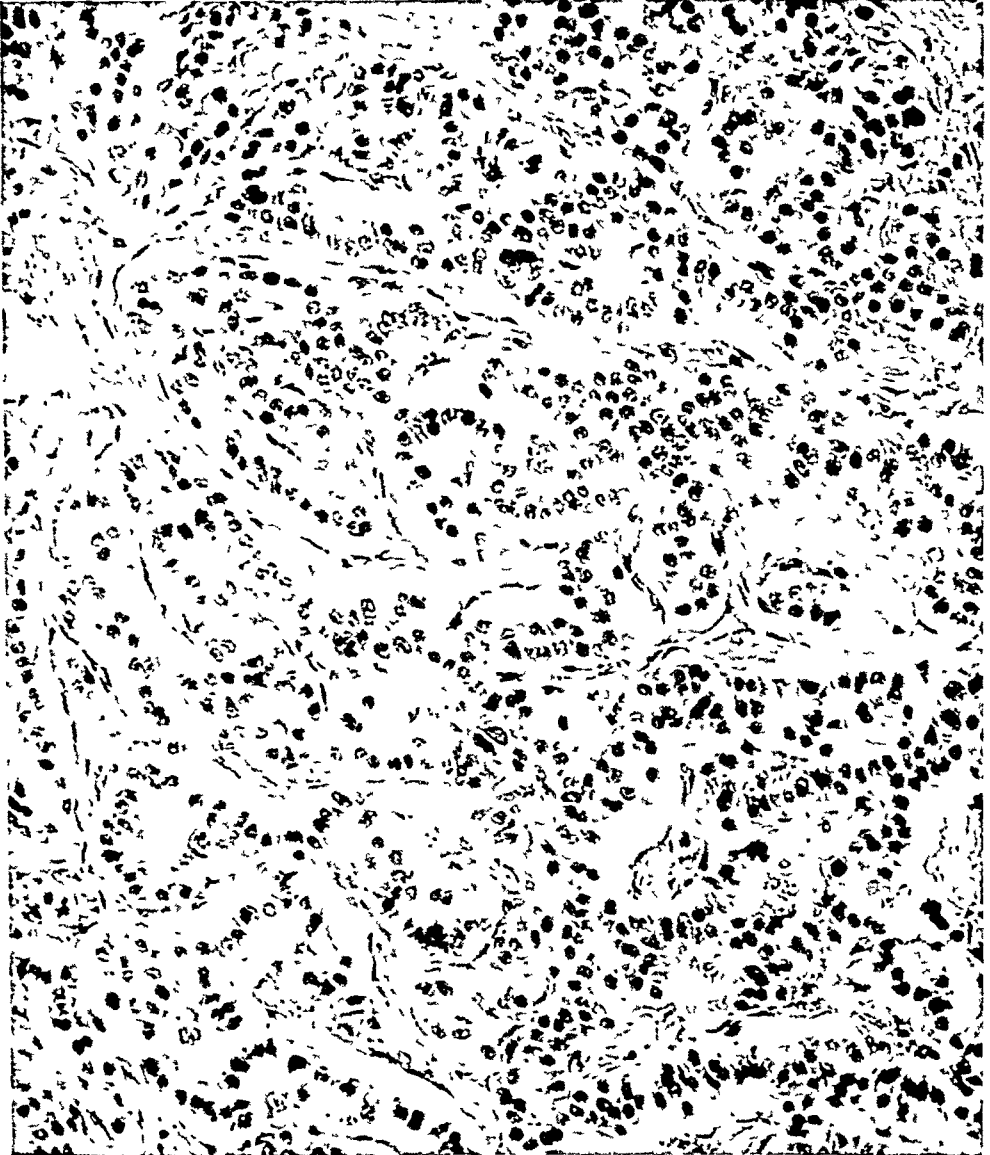


Fig. 4.—Microscopic appearance of the pancreatic adenoma in case 2.

There was a tiny infarct of the spleen, but the heart, lungs, kidneys, adrenals, prostate and pineal gland showed no essential abnormalities.

The major lesions were found in the pancreas, pituitary and esophagus. The nodule in the pancreas suggested an islet adenoma. It was composed of ribbon-like cords of columnar cells with a pale granular cytoplasm and regularly arranged oval nuclei (fig. 4). The cords were frequently one cell layer thick, with delicate

stroma and capillaries on both sides. In some places, double layers and papillary structures were present. In others there were glandular structures with basally placed nuclei. Solid masses of cells were present in some areas. The resemblance to islet tissue was definite. In some regions the cells showed pyknosis of the nuclei and disappearance of the cellular outline, with pale myxomatous, hyalin-like material deposited interstitially and perivascularly. Slight to severe fibrosis was present throughout, with fibrous septums transversing the adenoma. These were continuous with a thick fibrous capsule in which both adenomatous tissue and pancreatic acinar tissue were present. Ductlike structures were present in the adenoma and the capsule and at points along the capsule strongly suggested continuity with the adenomatous tissue, although serial sections failed to demonstrate this definitely. In one of these areas a few bizarrely shaped hyperchromatic nuclei and multinucleated cells were seen.

The pancreas elsewhere showed areas of fibrosis and fatty infiltration. The acinar tissue was not remarkable, but there were definite changes in the islets. Most of the islets appeared normal, but in a few areas in the head, near the adenoma, they were unusually large and numerous and showed a cellular structure unlike that of the other islets. They were composed of ribbons and cords of columnar cells and markedly resembled the tissue seen in the adenoma (fig. 5).

The pancreas, at first fixed in Jores^{6a} solution, was refixed and stained as in case 1. Although the preparations were not completely satisfactory, the normal-appearing islets showed both pale cells and cells with reddish granules in their cytoplasm. The former were considered to be of the alpha and the latter of the beta type. The cells of the adenoma uniformly showed a reddish granular cytoplasm similar to that seen in the beta cells.

The pituitary gland was fixed and stained as in case 1 and studied in five sagittal planes (Dr. Spark). The anterior lobe showed adenomatous hyperplasia of both the basophilic and the acidophilic cells, preponderantly the former. The chromophobe cells were reduced in number. Throughout the anterior portion were numerous large and small masses of basophilic cells in all stages of evolution. Some contained deeply stained granules; others were of the transitional type, with granules that stained light grayish blue. The cells were irregular in shape and size, and the nuclei tended to be large and vesicular. Binucleated basophilic cells were not uncommon, and vacuoles were large and numerous. No "colloid" basophils were found.

On the inferior surface of the anterior pole near the midline was a 4 by 1 mm. fibrosing basophilic adenoma, consisting of irregularly shaped nests of transitional basophils, separated by a moderate amount of loose stroma. At the same level were large groups of transitional basophilic cells undergoing necrobiosis, with small pyknotic nuclei, poorly defined cell membranes and marked loss of granules. At another level was a fairly well circumscribed mass, 1 mm. in diameter, of deeply granulated basophils with a few degenerated forms. In the posterior half of the anterior lobe the acidophils formed broad sheets of deeply granulated cells with only an occasional included basophil. The cells were typical, with normal nuclei, and showed little variation in size and shape. On the inferior surface near the anterior tip was a small oval adenoma composed of transitional acidophils and a few ripe basophilic elements.

There was relatively little follicle formation in the anterior lobe, and the connective tissue stroma was increased in a few areas.

6a. Mallory, F. B.: *Pathological Technique*, Philadelphia, W. B. Saunders Company, 1938.

The region of the pars intermedia contained a few moderate-sized cysts filled with colloid.

The posterior lobe showed a striking picture of massive infiltration by closely packed ripe basophil cells. These cells resembled the basophils of the anterior lobe in all respects except that only a few transitional forms were present, and

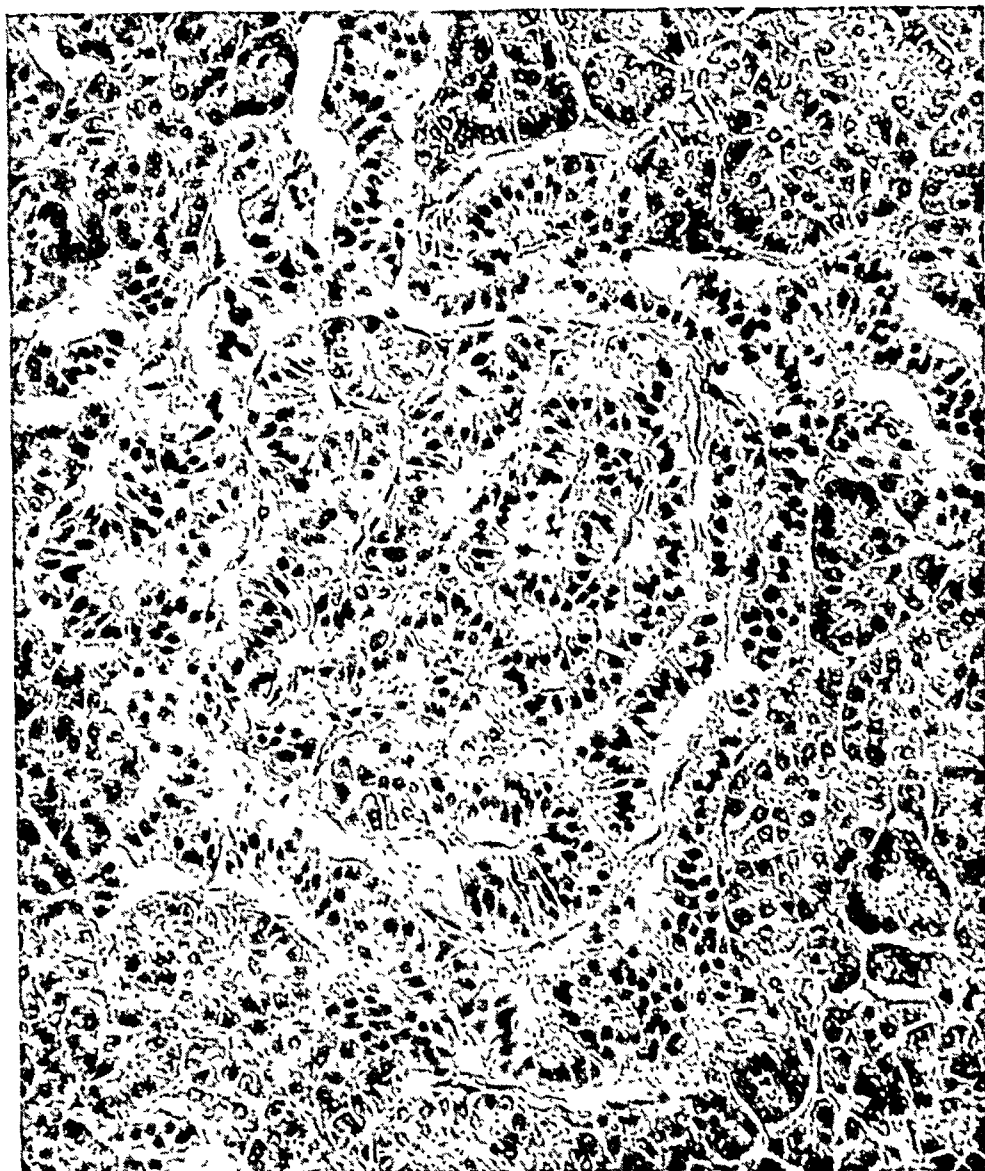


Fig. 5.—Microscopic appearance of a hyperplastic islet in the pancreas in case 2.

there was some follicle formation and colloid retention. From one half to three quarters of the pars nervosa was occupied by these basophilic elements (fig. 6). In some areas the capsule over the posterior lobe was also invaded by basophils. There was a moderate amount of pigment in the uninvaded portion.

The esophagus showed an extensive necrotic exudate replacing the mucosa and extending deeply into the muscle layers. There was edema, with considerable infiltration by inflammatory cells, chiefly lymphocytes, plasma cells and macrophages,

and a few polymorphonuclear leukocytes. Miscellaneous bacteria were present in the Gram preparation, with numerous larger forms showing branching hyphae and spores infiltrating the inflammatory membrane in palisade fashion, suggesting a fungus infection.

The cortical and hypothalamic regions of the brain were studied (Dr. Davison). The arterioles of the cortex showed proliferation of the endothelium, and the nerve cells showed the changes of ischemia. The nerve cells of the hypothalamic nuclei showed some poverty of iron pigment, while those of the paraventricular and supraoptic nuclei were swollen as well. The nerve cells of the nuclei tuberis proper were normal.

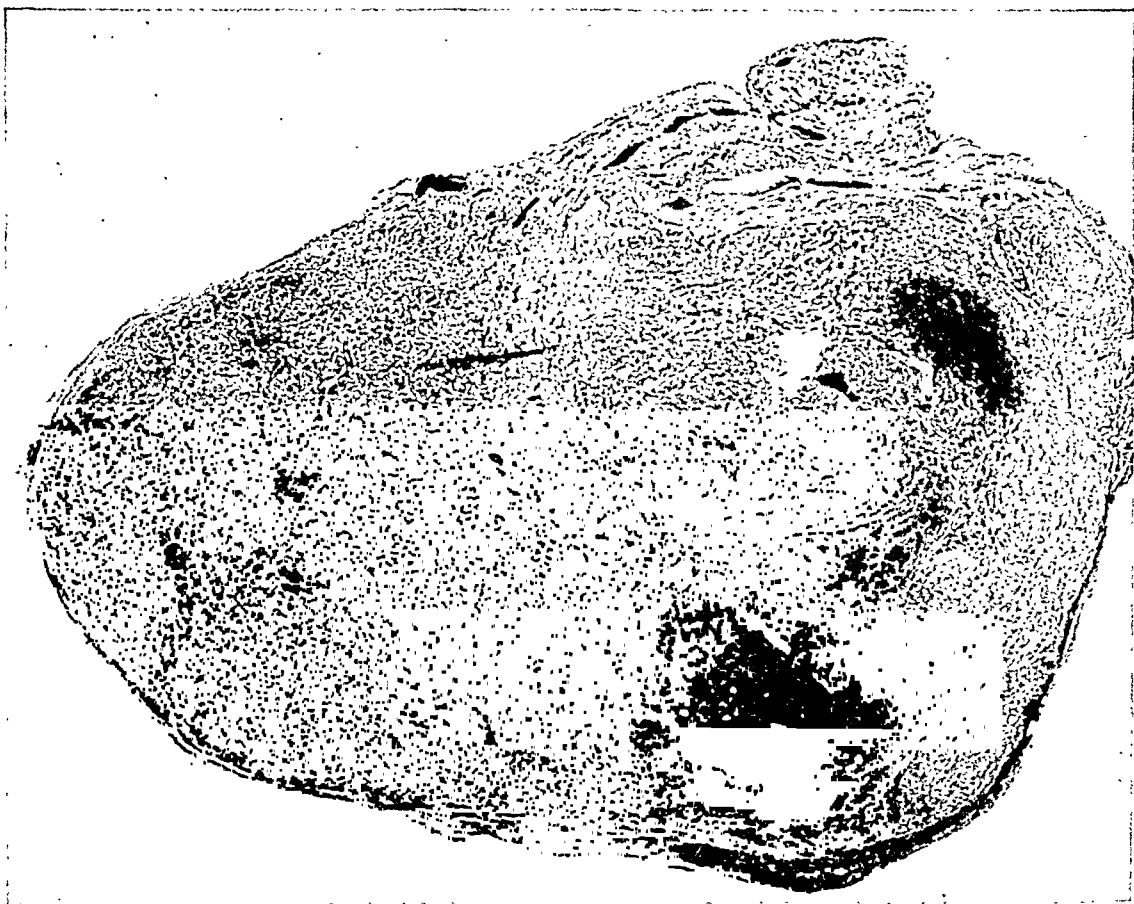


Fig. 6.—Microscopic appearance of the pituitary in case 2. Note the infiltration of the posterior lobe.

COMMENT

Clinical Observations.—One feature of interest was the dural hematoma confusing the clinical picture in case 2. This hematoma had been sustained, presumably, as a result of trauma during an earlier seizure. The possibility of such a complication in cases of this type should be borne in mind. In a case of Reiter's^{2m} previous injury of the skull and the presence of focal neurologic signs were similarly misleading. The failure of operative exploration to disclose a pancreatic adenoma as in case 1 has been reported. In this case, too, there was evidence of a disease of the central nervous system.

The effect of infection in temporarily ameliorating the severity of the hypoglycemic state in case 1 corroborates the observations of Blau, Reider and Bender³ on the same patient. They had observed, in addition, that the administration of typhoid vaccine, with fever, had a similar effect.

The opposite result, marked exacerbation of the hypoglycemia following irradiation of the pituitary region, is interesting in view of the well known effects of hypophysectomy on sensitivity to insulin.

One of the most significant of all the changes noted in chronic hypoglycemic patients of this type, one that has been commented on by many observers, is the marked progressive obesity. This was present in both of our cases and may be due to the high caloric diets given therapeutically or taken spontaneously by such patients. It is tempting to speculate on this phenomenon as a clue in unraveling the genesis of some types of obesity. Falta many years ago suggested that obesity may be related to overactivity of the pancreas, and in this type of case such a possibility has become an actuality. This might be considered a true endocrinal form of obesity with a definite anatomic and physiologic basis in the islet tumor and the hypoglycemia.

Among others, Massa,⁷ Kup,⁸ Harris⁹ and Lichtwitz¹⁰ have in recent years discussed this relationship. Ogilvie¹¹ found more islet tissue than in the controls in the pancreas of the obese subject and hypoglycemic sugar tolerance curves in one third of his cases of early obesity. The finding of increased sugar tolerance in obesity has again been reported by Leites and Agaletzkaia.¹² Insulin has been used therapeutically to increase weight for some time, although there have been some contradictory studies. Mackay and Callaway¹³ recently produced obesity in animals by the use of protamine insulin, and I have confirmed this observation.¹⁴

Pathologic Observations.—The adenomas in the pancreas were quite typical. The histologic characters of such tumors have been studied in detail by O'Leary and Womack¹⁵ and by Laidlaw.¹⁶ The ribbon-

7. Massa, M.: *Gior. di clin. med.* **10**:679, 1929.

8. Kup, J.: *Endokrinologie* **6**:102, 1930.

9. Harris, S.: *Am. J. Digest. Dis. & Nutrition* **2**:557, 1935.

10. Lichtwitz, L.: *Pathologie der Funktionen und Regulationen*, Leiden, A. W. Sijthoff's Uitgeversmaatschappij N. V., 1936.

11. (a) Ogilvie, R. F.: *J. Path. & Bact.* **37**:473, 1933. (b) Ogilvie, R. F.: *Quart. J. Med.* **4**:345, 1935.

12. Leites, S., and Agaletzkaia, A.: *Acta med. Scandinav.* **89**:199, 1936.

13. Mackay, E. M., and Callaway, J. W.: *Proc. Soc. Exper. Biol. & Med.* **36**:406, 1937.

14. Unpublished data.

15. O'Leary, J. L., and Womack, N.: *Arch. Path.* **17**:291, 1934.

16. Laidlaw, F. G.: *Am. J. Path.* **14**:125, 1938.

like cords of cells resembling those described by MacCallum ¹⁷ and Cecil ¹⁸ in islet hypertrophy are interesting particularly in view of the changes found in the other islets in case 2. Many of the islets near the adenoma revealed a histologic picture quite like that of the adenoma itself, and exhibited these ribbons. This change suggests a more diffuse islet hyperplasia with adenoma formation rather than the occurrence of a single localized tumor. It is unfortunate that in case 1 the pancreas was not sufficiently well preserved for studies of the islets throughout the organ. Several observers have noted that in cases of hypoglycemia with islet adenoma the pancreatic islets show such changes. Cases in which multiple adenoma or even diffuse adenomatosis was present have been reported. Specific granules were demonstrated in the cells of both adenomas. As other studies have shown, the granules resembled beta granules, although they were not quite typical.

The changes in the pituitary gland were marked. The massive basophil infiltration of the posterior lobe in the one case and the relatively slight infiltration in the other are possibly of less significance than the marked adenomatous hyperplasia in the anterior lobe.

Similar changes have been described in other cases of islet adenoma with hypoglycemia. Terbrüggen's ¹⁹ case showed two "chief" cell adenomas. In the case reported by Rienhoff and Lewis ²⁰ an excess of basophils was observed and two tiny adenomas without specific granules but "staining more deeply with ordinary stains." There was also basophilic infiltration of the posterior lobe. In the case reported by Malamud and Grosh ²¹ eosinophilic hyperplasia and a small basophil adenoma were noted.

Another type of change, which is considerably more complex, has been seen in 2 cases. In a case studied by Kalbfleisch ²² there were multiple pancreatic adenomas. One of these had been surgically removed because of progressing hypoglycemia in spite of the presence of a Fröhlich syndrome complex and roentgen evidence of damage to the sella turcica. At necropsy the other islet tumors were found. There was a chromophobe adenoma of the pituitary extending out of the sella. The compressed pituitary showed slight basophil infiltration of the posterior lobe, and in the anterior lobe the eosinophils were more numerous than the basophils and the latter more numerous than the chromophobes. The parathyroids showed changes suggesting chief cell adenoma, and softness of the bones was noted. This case is reminiscent of the one

17. MacCallum, W. G.: *Am. J. M. Sc.* **133**:432, 1907.

18. Cecil, R. L.: *J. Exper. Med.* **13**:595, 1911.

19. Terbrüggen, A.: *Beitr. z. path. Anat. u. z. allg. Path.* **88**:37, 1932.

20. Rienhoff, W. F., Jr., and Lewis, D.: *Bull. Johns Hopkins Hosp.* **54**:386, 1934.

reported by Lloyd,²¹ in which the pituitary tumor dominated the clinical picture. A 22 year old obese woman with male distribution of hair and hypogenitalism died after operation. She showed glycosuria once. At necropsy multiple nodules composed of islet tissue and many large islets were found in the pancreas. There was an extrasellar chromophobe adenoma of the pituitary. The gland was reported as otherwise normal. The parathyroids were large and showed chief cell adenomas.

The pituitary changes in the 3 cases first mentioned and in the 2 cases reported in this paper certainly indicate active disease of some sort, but their exact significance must await further study. It is once more tempting to consider these changes as possibly related to the changes found in the pituitary in cases of obesity. Zeynek²² and Muller,²³ among others, found an increase in the number of basophils in the pituitary and the formation of basophil and transitional cell adenomas in persons suffering from obesity to a greater extent than in nonobese persons. Spark²⁴ in his laboratory confirmed these observations (unpublished).

Changes in the central nervous system have occupied the attention of many workers. Various degenerative processes have been described by Terbrüggen,¹⁹ Baker and Lufkin,²⁵ Moersch and Kernohan,²⁶ Malamud and Grosh²⁴ and many others. Experimental hyperinsulinism and the changes in the central nervous system have also been investigated, a recent report being that of Weil, Liebert and Heilbrunn.²⁷ In the cases reported in this paper the changes found were consistent with their observations.

Multiple adenomas in many endocrine glands and viscera, such as have been found in some of the reported cases and in my case 1, have been taken by Lichtwitz¹⁰ and others to indicate that a central neuro-humoral mechanism of some sort may be primarily at fault. In the presence of lesions in the central nervous system, such a possibility deserves consideration.

In my case 1, in which death occurred while the patient was in hypoglycemic shock, the intense cyanotic congestion of the viscera resembled that reported in a case of Ziskind's. The esophagitis and

21. Lloyd, P. C.: *Bull. Johns Hopkins Hosp.* **45**:1, 1929.

22. Zeynek, E.: *Frankfurt. Ztschr. f. Path.* **44**:387, 1933.

23. Muller, M.: *Endokrinologie* **18**:114, 1936.

24. Spark, C.: Unpublished data.

25. Baker, A. B., and Lufkin, N. H.: *Arch. Path.* **23**:190, 1937.

26. Moersch, F. P., and Kernohan, J. W.: *Arch. Neurol. & Psychiat.* **39**:242, 1938.

27. Weil, A.; Liebert, E., and Heilbrunn, G.: *Arch. Neurol. & Psychiat.* **39**:467, 1938.

gastritis as possible complications of the hypoglycemia and the intake of large amounts of carbohydrate are somewhat interesting.

SUMMARY

Two cases of chronic hypoglycemia, in each of which an islet adenoma of the pancreas was found at necropsy, are reported. In both cases marked hyperplasia of the basophilic and eosinophilic cells of the pituitary was noted. In one case hyperplastic changes in the islets of Langerhans suggested that the adenoma might be related to a generalized change in the islet apparatus.

The significance of the changes in the pancreas and pituitary are discussed with particular reference to the obesity that developed in both patients.

OBSERVATIONS ON LESIONS PRODUCED IN ARTERIES OF DOGS BY INJECTION OF LIPIDS

LIPIDS INJECTED: HUMAN FAT, FATTY ACIDS, SOAPS
AND CHOLESTEROL

OSCAR O. CHRISTIANSON, M.D.
UNIVERSITY, ALA.

Focal lesions of arteries have been produced experimentally in several ways. Schilling¹ produced such lesions in the arteries of rabbits by searing and tearing the walls and by stripping off the adventitia. Ssolowjew produced them by stretching² and cauterizing³ the arteries. Anitschkow⁴ and Ssolowjew traumatized arteries of rabbits fed a high cholesterol diet in order that they might determine the effect of mechanical injuries on the deposition of lipids.

There apparently has been no investigation of the effects produced in the walls of arteries by injections of various known lipids. Studies have been made, however, of lesions produced in other tissues by injections of fat materials. Wail⁵ injected lipids into the subcutaneous tissues of rabbits and by microchemical studies followed the changes in the composition of the fats as well as the tissue reactions at intervals after the injections. Hirsch⁶ studied lesions occurring in the lungs of rabbits after several intravenous injections of lipids. Hagerty⁷ produced lesions in the kidneys of rabbits and dogs, similar to those seen in diffuse glomerulonephritis, by injecting lipids into the renal arteries.

The tissue lesions which I have studied were produced by injecting lipids into arterial walls in dogs. These lipids consisted of human fat alone or human fat mixed with one or more of the following ingredients: oleic acid, stearic acid, cholesterol or these acids neutralized with

From the John Jay Borland Fellowship for Clinical Research of the Henry Baird Favill Laboratory of St. Luke's Hospital, Chicago, and the Department of Pathology of the University of Alabama Medical School, University, Ala.

1. Schilling: *Verhandl. d. deutsch. path. Gesellsch.* **20**:154, 1925.
2. Ssolowjew, A.: *Ztschr. f. d. ges. exper. Med.* **69**:94, 1930.
3. Ssolowjew, A.: *Virchows Arch. f. path. Anat.* **283**:213, 1932.
4. Anitschkow, N.: *Beitr. z. path. Anat. u. z. allg. Path.* **59**:306, 1914.
5. Wail, S. S.: *Virchows Arch. f. path. Anat.* **245**:219, 1923.
6. Hirsch, E. F.: (a) *Arch. Path.* **21**:765, 1936; (b) **25**:35, 1938.
7. Hagerty, C. S.: *Arch. Path.* **25**:24, 1938.

aqueous solutions of sodium hydroxide or calcium hydroxide. A comparative study was made of the tissue reactions induced, at various intervals of time.

MATERIALS AND METHODS

Sterile solutions of the lipids were injected into the media and subintimal tissues of the abdominal aorta and femoral arteries through a 26 gage hypodermic needle. Frequently the lumen of the vessel was entered by the needle and a small hemorrhage resulted. In control experiments to determine the tissue reactions induced by the extravasated blood, the arteries were pierced several times. Eighty dogs, anesthetized with pentobarbital sodium for the operation, were killed with ether. The arterial segments were fixed in solution of formaldehyde U. S. P. (1:10) and in Zenker's solution. Those fixed in the formaldehyde solution were sectioned by the freezing method, stained with scarlet red and counterstained with hematoxylin. Sections were mounted unstained and examined with a micropolariscope for cholesterol esters. Sections from each vessel were examined by the Schultz⁸ method to detect minute quantities of cholesterol. Tissues fixed in Zenker's solution were embedded in paraffin, cut, and stained with hematoxylin and eosin and with phosphotungstic acid-hematoxylin.

MICROSCOPIC OBSERVATIONS AT SITES OF INJECTION OF TEST SUBSTANCES

Human Fat.—At the end of the first day the region about the fat in the media and adventitia was infiltrated by many polymorphonuclear leukocytes. Usually there were marked fatty changes of the surrounding muscle fibers. The internal elastic lamina was slightly swollen. The capillaries of the media were dilated. The intimal lining cells were swollen and partly desquamated. In five days a vascular and edematous granulation tissue had formed. In this were masses of large mononuclear cells, many containing fine fat globules. The cellular reaction was most marked at the end of the first week. The lesion consisted mainly of chronic exudate cells, a few fibroblasts and an occasional polymorphonuclear leukocyte. The reaction was more marked in the adventitia than in the media. When fat was injected into the media, an intimal plaque usually formed within five to seven days. Most of these plaques were directly continuous through a destroyed portion of the internal elastic lamina into the fibroblastic tissues about the fat in the media. The new plaques consisted of many oval and round cells that seemed to proliferate locally. In the older lesions the cells were spindle shaped and separated by abundant collagenous fibers.

After twenty-eight days most of the fat was absorbed from the media. The remaining fat was surrounded by a fibroblastic tissue with many mononuclear cells. The lipid contained a greater proportion of crystalline fat than at the time of injection. In eight to ten weeks the crystals and granular fat debris often lay free in close proximity to large mononuclear cells. In other places they were surrounded by dense acellular scar tissue. Some of the old medial scars were scarcely visible; the internal elastic lamina remained interrupted, but there was often reduplication or splitting near its broken ends. In two hundred and twelve days the changes were similar except that only traces of fat remained, and the scars were smaller.

8. Schmorl, G.: Die pathologisch-histologischen Untersuchungsmethoden, ed. 16, Berlin, F. C. W. Vogel, 1934, p. 180.

Human Fat Neutralized Over an Aqueous Solution of Sodium Hydroxide.—The lesions resembled those caused by non-neutralized fat (fig. 1*A*). The rate of absorption of the fat appeared slightly more rapid after neutralization.

Human Fat with 5 Per Cent Cholesterol.—This is approximately a saturated solution at 37.5 C. The acute inflammatory changes in the first few days were like those caused by fat alone, but after the first week the granulation tissue was more extensive and cellular. Many foreign body giant cells and clusters of large mononuclear cells were about acicular cholesterol crystals or fat droplets. The healing process was slow. Even after ten weeks some arteries had a considerable infiltrate of large mononuclear cells and foreign body giant cells about an occasional cholesterol crystal. No foreign body giant cells were noted in the media. In a healed lesion of one artery were two long acicular cholesterol crystals embedded in a small dense scar of the media just below the intact internal elastic lamina. At this level there was also a small intimal fibrous plaque. The scars were slightly larger and denser than those induced by human fat.

*Human Fat with 16 Per Cent Oleic Acid.*⁹—In the first few days slight necrosis and hemorrhage appeared about the lipid. In five to fourteen days the lesions were like those produced by human fat combined with cholesterol except that the foreign body giant cells were not so numerous (fig. 1*B*). The fat was absorbed rapidly, and after twenty-eight days most of the arteries contained only traces of fat globules, a few fat crystals and granular debris. Although the initial tissue reaction was marked, a healed lesion resulted considerably sooner than after the injection of fat alone or combined with cholesterol.

Human Fat, 16 Per Cent Oleic Acid and 5 Per Cent Cholesterol.—This was not a saturated solution of cholesterol at 37.5 C. All lesions were observed one hundred and fifty-five to one hundred and seventy-eight days after the injection. There were small scars with occasional large mononuclear cells. In one artery a cluster of small acicular cholesterol crystals was embedded in a dense acellular scar of the adventitia.

Human Fat and 16 Per Cent Oleic Acid Neutralized Over an Aqueous Solution of Calcium Hydroxide.—The tissue response in the first two to three weeks was similar to that induced by mixtures of human fat and oleic acid. Many foreign body giant cells and mononuclear cells were about small free-lying granules of calcium oleate. The lipids, however, were absorbed more slowly, and the lesions were more chronic than those caused by the aforementioned solution (fig. 2*A*).

Human Fat and 16 Per Cent Stearic Acid.—In one artery examined one hundred and eighty-four days after the injection of the fat a small scar of the media remained. Overlying this was a small intimal plaque of swollen and granular collagenous fibers (fig. 2*B*).

Human Fat, 16 Per Cent Oleic Acid and 5 Per Cent Cholesterol Neutralized Over an Aqueous Solution of Calcium Hydroxide.—These arteries were examined thirty-seven days and one hundred and forty-eight days after the injection. The tissue response resembled the others. In the older lesion a small cluster of cholesterol crystals was in a scar of the adventitia.

9. The oleic acid was prepared by P. J. Hartsuch in the Henry Baird Favill Laboratory of St. Luke's Hospital, Chicago. This purified acid had an iodine number of 99.4 and an acid number of 198.4. The percentage of oleic acid was approximately 92 and of linoleic acid 8.



Fig 1.—*A*, photomicrograph of the femoral artery of a dog thirteen days after human fat neutralized by sodium hydroxide had been injected into the media; $\times 124$. Note the small intimal plaque, the intact internal elastic lamina and the granulation tissue about the injected fat. *B*, photomicrograph illustrating the changes in the wall of the femoral artery of a dog fourteen days after a mixture of human fat and 16 per cent oleic acid had been injected into the media; $\times 124$. The internal elastic lamina is interrupted and an intimal plaque is continuous into the fibroblastic tissue of the media.

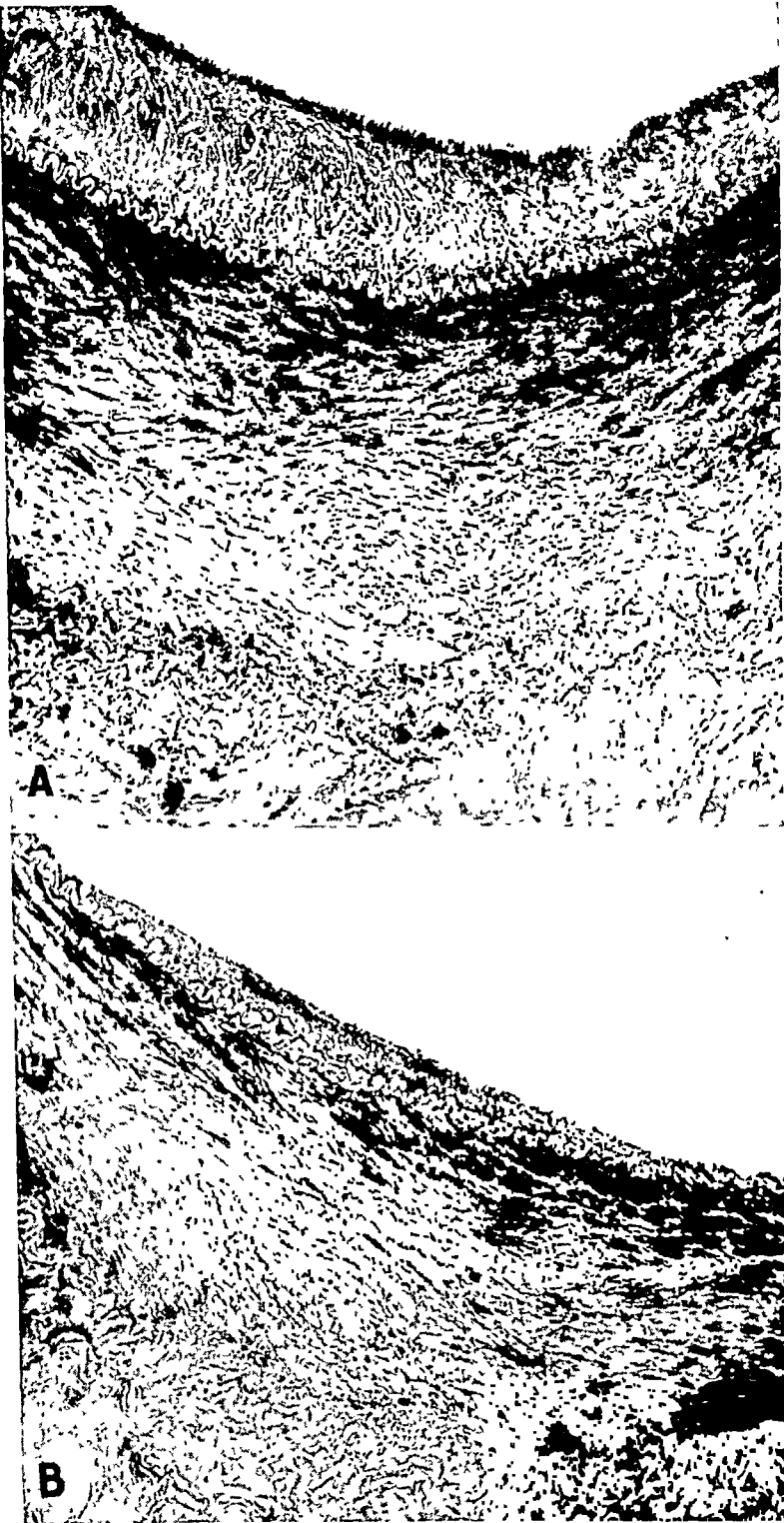


Fig. 2.—*A*, photomicrograph of a large intimal plaque in the femoral artery of a dog; $\times 124$. The wall of the artery had received an injection of an emulsion of human fat and 16 per cent calcium oleate twenty-eight days before death. Note the black deposits of calcium oleate in the medial portion of the adventitia. *B*, photomicrograph illustrating the lesions in the wall of a femoral artery into which a mixture of human fat and 16 per cent stearic acid was injected one hundred and eighty-four days before death; $\times 124$. Note the granular and swollen appearance of the intimal plaque. In the media is a small scar.

Human Fat, 16 Per Cent Stearic Acid and 5 Per Cent Cholesterol Neutralized Over an Aqueous Solution of Calcium Hydroxide.—These lesions were like those produced by a mixture of human fat and oleic acid neutralized over calcium hydroxide.

Control Experiments.—Hemorrhage and trauma were produced by piercing the wall of the artery with a hypodermic needle. Twenty-three arteries were examined one to seventy-seven days later. The extravasated blood was absorbed rapidly; no appreciable tissue changes resulted.

REVIEW OF OBSERVATIONS

All fat mixtures provoked considerable tissue reaction about the site of injection. The resulting granuloma was maximal in one to two weeks. Fatty acids or calcium soaps added to the human fat injected augmented the acute and chronic inflammatory changes. Cholesterol or calcium soaps added to the fat produced more chronic lesions and a larger scar.

Microscopically, the tissue lesions caused by a mixture of human fat and fatty acids were more severe in the first two weeks than those caused by fat alone. But this mixture was absorbed more rapidly and the lesions healed sooner than when only human fat was injected. If the fat contained calcium soaps, similar intense tissue reactions occurred in the first few days. The calcium soaps were slowly absorbed, however, and more chronic lesions resulted than after the injection of a combination of fatty acids and human fat. When the fat contained a small quantity of cholesterol, similar chronic inflammatory lesions resulted. Human fat alone did not evoke foreign body giant cells. Mixtures of fat with fatty acids, calcium soaps and cholesterol produced inflammatory exudates containing many of these cells.

The absorption of fat began in four days or less; in many arteries most of it was absorbed within four weeks. After much fat was absorbed, the proportion of crystalline fat in the globules increased. The crystals were surrounded by clusters of large mononuclear cells and usually small foreign body giant cells. Often with cholesterol long acicular crystals were found in recent and old lesions. In the latter, crystals of fat and cholesterol were embedded in a dense acellular scar. Most of the cholesterol was absorbed gradually, for the Schultz test⁸ became less strongly positive the older the lesion.

The inflammatory response about the injected lipid was consistently more marked in the adventitia than in the media. Within forty-eight hours many large mononuclear cells, some laden with fat droplets, were observed about fat injected into the media. They were near dilated capillaries of the media; they probably came from the blood stream. Foreign body giant cells were observed only in the adventitia. In several weeks the scars of the media became inconspicuous and

often could not be identified without the aid of specific stains. The phosphotungstic acid-hematoxylin preparations demonstrated some interruptions of the elastic tissue and replacement of the smooth muscle by a small amount of fibrous tissue.

Fat mixtures injected into the media in 38 arteries produced intimal fibrous plaques in 32 and a disruption of the internal elastic lamina in 23. No secondary fatty changes of these plaques were noted. A few plaques over five months old were nearly acellular and consisted of swollen granular collagenous fibrous tissue that had a slightly mucoid appearance. No definite evidence of deposition of calcium was noted in the healed lesions. Often a little dense granular debris remained which may have been calcium granules or remnants of insoluble fat crystals.

COMMENT

Several interesting observations were made during this study of the lesions resulting in the walls of arteries from injections of known lipids. The character of the lesions seemed to depend on several factors, as discussed by Hirsch.^{6b} This dependence concerned mainly the degree of acidity and the solubility of the injected lipids. Fatty acids produced intense acute inflammatory reactions, but the lipids were absorbed readily; therefore, the lesions healed rapidly. Calcium soaps and cholesterol components which were only slightly soluble in human fat and insoluble in tissue fluids often precipitated. As a result, they were absorbed slowly and produced extensive chronic inflammatory changes. The lesions were consistently more marked in the adventitia than in the media.

Lipids injected into the media not only provoked acute and chronic inflammatory reactions in the immediate vicinity but caused degeneration of the overlying internal elastic lamina and development of intimal plaques (fig. 1 *B*). Several investigators produced similar lesions by mechanically injuring the media. Whether intimal plaques would develop without injury to the elastic tissue is difficult to say. In a few specimens where the plaques had formed in the media at the level of the injected fat, the internal elastic lamina appeared unaltered (fig. 2 *A*). Occasionally the lesion of the media caused flattening of the internal elastic lamina without producing an intimal plaque. Cowdry¹⁰ stated that intimal plaques occur spontaneously in animals, especially in dogs; therefore, the assumption that they are caused by injury of an artery may be erroneous. Such plaques were observed occasionally in my experiments, but only in the aorta. The majority of the intimal plaques produced were directly continuous with the medial lesions or in close proximity to the injected fat in the media.

10. Cowdry, E. V.: *Arteriosclerosis: A Review of the Problem*, New York, The Macmillan Company, 1933.

In the discussion of the factors involved in the production of arteriosclerosis in man much emphasis has been placed on the role of cholesterol. Anitschkow¹¹ stated that without cholesterol arteriosclerosis could not occur. In my experiments even human fat devoid of cholesterol produced degenerative and inflammatory changes and scars of the media, splitting or rupturing of the internal elastic lamina, and intimal plaques. The addition of cholesterol tended to produce only a more extensive and chronic lesion. Leary¹² suggested that the agent responsible for the stimulation of a marked growth of connective tissue in youths is some soluble product of cholesterol metabolism and not the precipitated cholesterol crystals. However, he believed that in old age when atheromas contain a large quantity of free cholesterol crystals the cholesterol material may stimulate connective tissue growth. Since the cholesterol probably was brought to these regions with solvent fats, it may be that these fats, through a process of hydrolysis or oxidation, liberate fatty acids which alone or in the form of their soaps stimulate the formation of fibroblastic tissues or of an intimal plaque.

During this investigation the absorption of cholesterol occurred slowly, and often large crystals remained embedded in a dense scar. Crystals occasionally were found near remnants of solvent fat globules. Anitschkow observed this relationship of cholesterol crystals and fat globules in healing arteriosclerotic lesions produced in rabbits by a diet high in cholesterol. He thought the fat had infiltrated to help dissolve the cholesterol. Perhaps the fat he observed was a residue of the solvent lipid that had conveyed the cholesterol to the lesion.

Zinserling¹³ described primary or spontaneous intimal plaques in dogs. In old dogs they usually were associated with a frayed internal elastic lamina. He observed primary fatty changes of the muscle fibers in the inner third of the media and deposition of fat and cholesterol in the ground substance. Many foam cells were present, and the lesion was surrounded by fibrous tissue. The adjacent internal elastic lamina was frayed; usually there was an intimal plaque. He stated that the usual arteriosclerotic lesion in the dog is a secondary fatty change of the spontaneous intimal plaque; this lesion is combined often with changes in the media. He thought that in some way the plaques favored infiltration of lipids directly from the blood stream. To explain the frequently associated fatty changes in the media he supposed that the fat was carried from the intimal plaque to the media by the lymph channels and absorbed.

11. Anitschkow, N.: *Virchows Arch. f. path. Anat.* **249**:73, 1924.

12. Leary, T.: *Arch. Path.* **17**:453, 1934.

13. Zinserling, W. D.: *Beitr. z. path. Anat. u. z. allg. Path.* **88**:241, 1932.

There is considerable evidence, however, that the medial lesions in arteriosclerosis may precede the intimal. Duff¹⁴ held that cholesterol compounds from the blood are only secondary in the formation of arteriosclerotic lesions. He regarded some alteration or lesion of the media as primary and stated that this produced changes in the sub-endothelial tissues favorable to infiltration of lipids from the blood. His assumption was based partly on a study of lesions in the aortas of rabbits. He noted close to the internal elastic lamina spontaneous focal necrosis of muscle fibers before any intimal changes occurred. In the more advanced lesions anisotropic fat deposits were noted in the intercellular ground substance. He observed that in rabbits fed a cholesterol-rich diet the spontaneous lesions became infiltrated with anisotropic lipids much more rapidly than other parts of the aorta. He suggested that sometimes this degeneration of the media produced intimal plaques, or the subendothelial swelling which facilitated infiltration of lipids from the blood. This assumption is substantiated by Ssolowjew² and others, who noted that mechanical injury to the blood vessels predisposed to the deposition of lipids in these regions in rabbits fed high cholesterol diets.

Leary¹⁵ studied the early changes in the atheromatous plaques of human arteries. He described a mucoid degeneration of the swollen ground substance of the subendothelial tissues. He expressed the belief that this change may be due to a slight thyroid insufficiency and that it facilitates the deposition of fat. In my experiments some intimal plaques degenerated after five months and had a swollen mucoid appearance.

My studies demonstrate that lipids in the wall of an artery, even those without cholesterol, produce inflammatory lesions of the media and the intima similar to those observed frequently in human arteriosclerosis. It seems that local injury of the media may be the first change in the formation of an atheromatous plaque. The injured region then becomes infiltrated with lipids which in turn stimulate the proliferation of fibroblastic tissue. An important effect of this medial lesion is an alteration of the overlying intima which predisposes to infiltration and deposition of lipids in the subendothelial tissues.

SUMMARY

Lesions were produced in arteries of dogs by injecting into the media human fat, alone or mixed with fatty acids, calcium soaps or cholesterol. The severity and chronicity of the lesions varied with the acidity and speed of dispersal of the fat mixture. Human fat and fatty

14. Duff, L.: Arch. Path. **22**:161, 1936.

15. Leary, T.: Arch. Path. **21**:419, 1936.

acids produced marked acute inflammatory lesions, which healed rapidly because the lipids absorbed readily. Human fat mixed with calcium soaps or cholesterol was absorbed slowly and caused a chronic lesion. Often fat and cholesterol crystals separated, became surrounded by chronic inflammatory exudates, including foreign body giant cells, and finally were embedded in dense acellular scars.

In the formation of arteriosclerotic lesions in man the infiltrating lipids as well as cholesterol may be important in producing fibrous tissue. The products of hydrolysis and of oxidation of the fats are probably responsible for the tissue changes.

Most of the medial lesions produced disruption or splitting of the internal elastic lamina and development of intimal plaques. Thus intimal lesions were secondary to medial lesions simulating the early changes described in arteriosclerosis of man. Injuries of the media may be important in the production of secondary intimal changes which predispose to the deposition of lipids in the early lesions of arteriosclerosis.

Case Reports

LEFT INFERIOR VENA CAVA

BENJAMIN A. GOULEY, M.D., AND PHILIP D. GILBERT, M.D., PHILADELPHIA

The congenital lesion of the heart herein reported is so unusual that we deem it worthy of record. It represents a malformation of the venoauricular structure, the inferior vena cava following the left side of the body and emptying into the left auricle; there was an associated persistent ostium primum. We are unaware of any report of a similar case in Anglo-American literature.

REPORT OF CASE

A full term Negro girl was born Sept. 3, 1936, weighing 9 pounds 6 ounces (4,252 Gm.). The child progressed satisfactorily for eight days, when cyanosis and rapid gasping respiration suddenly developed. There was marked enlargement of the heart, with widening of the area of supracardiac dulness, and a loud systolic murmur was heard over the entire precordium, having its maximum intensity at the pulmonic area. The electrocardiogram showed right axis deviation, which at this age had no special significance. After a temporary response to oxygen therapy, the infant died on the ninth day.

Necropsy revealed passive congestion of the viscera and congenital heart disease. The right ventricle was greatly enlarged; the cardiac apex, formed entirely by the right ventricle, pointed to the right. The left ventricle was comparatively small, only a small portion of it appearing anteriorly.

With the heart in situ, other striking features were noted. One was a structure hanging down over the right lateral ventricular border like a large saddle bag, an extraordinary right auricular appendage. The left auricular appendage was slightly enlarged and globular. Four large vessels arose from the base of the heart. The superior vena cava lay partially posteriorly on the right and was largely obscured. It was normal in all respects, emptying into the right auricle. The three large vascular trunks which occupied the anterior aspect of the supracardiac area were, as appeared from their size and location, abnormal: (1) The systemic aorta, which veered slightly to the right, gave rise at the arch to the three great arteries and after progressive narrowing connected by a patent ductus arteriosus with (2) a very large pulmonary artery, which sent a branch to each lung and continued as the thoracic aorta; (3) a large trunk, forming the left border of the widened supracardium, which extended upward from the left auricle, paralleled the thoracic aorta, arched posteriorly at the same level and continued down along the left side of the aorta into the pelvis as the inferior vena cava, receiving the renal veins and being formed by the junction of two iliac veins.

From the Departments of Pathology and Cardiology of the Philadelphia General Hospital.

Section of the heart revealed a persistent ostium primum. The right auricle was enlarged, and its elongated appendage almost reached the apex of the heart. Hepatic veins emptied into the right auricle, and normal pulmonary veins emptied into the left. The inferior vena cava entered the upper lateral portion of the left auricle almost at the junction of the latter with the auricular appendage. Its entrance was unguarded by any valve formation.

The hepatic veins were anomalous. Rather large veins extended upward over the anterior aspect of the liver and passing through the diaphragm reached the right auricle (fig. 1). Another smaller vein came up from the posterior aspect of the liver, at approximately the same level as the anterior vein, and joined the latter on entering the right auricle. These hepatic veins had no connection with

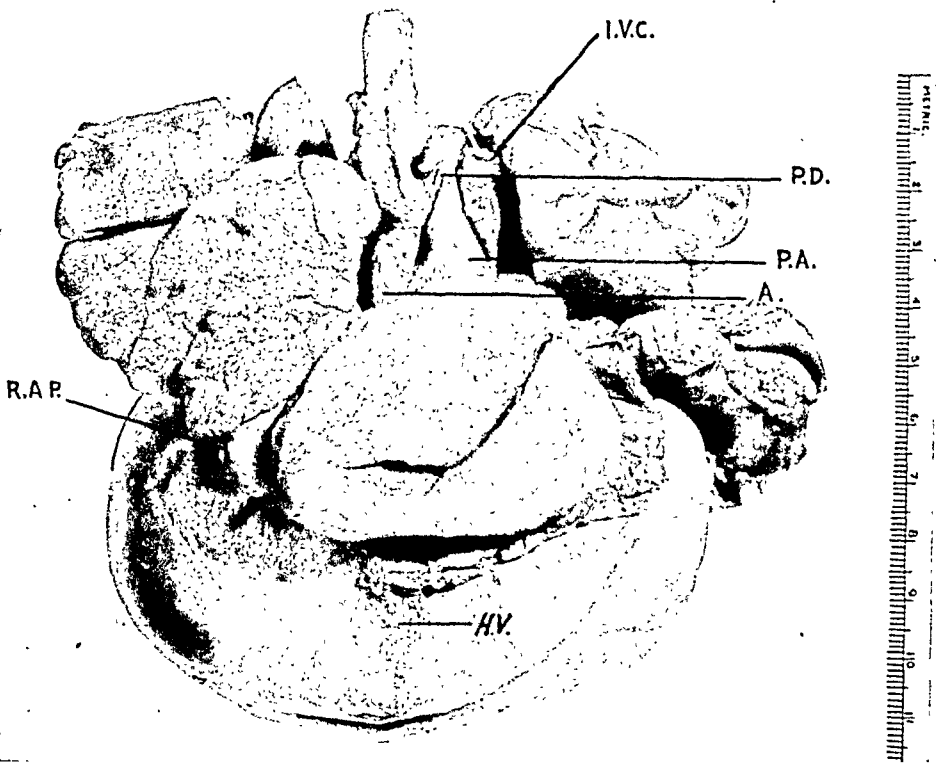


Fig. 1.—Heart, great vessels, lungs and liver. The heart turns to the right (dextrocardia). Extending down almost to the cardiac apex is a rounded baglike structure, the right auricular appendage (*R. Ap.*). The superior vena cava is obscured by the right lung, but three large vascular trunks are seen at the base of the heart, namely, the aorta (*A.*), the pulmonary artery (*P. A.*) and the inferior vena cava (*I. V. C.*); the last enters the left auricle. A patent ductus arteriosus (*P. D.*) connects the narrowed aorta with the pulmonary artery, which below this point (obscured) has given off arterial branches to both lungs. Aberrant hepatic veins from the anterior surface of the liver (one is opened, *H. V.*) course upward through the diaphragm to reach the right auricle.

any other venous system as far as could be determined. The liver itself was of unusual shape, its anterior aspect having a marked convexity; the lower border was without a definite edge, the anterior and inferior aspects rounding into each other.

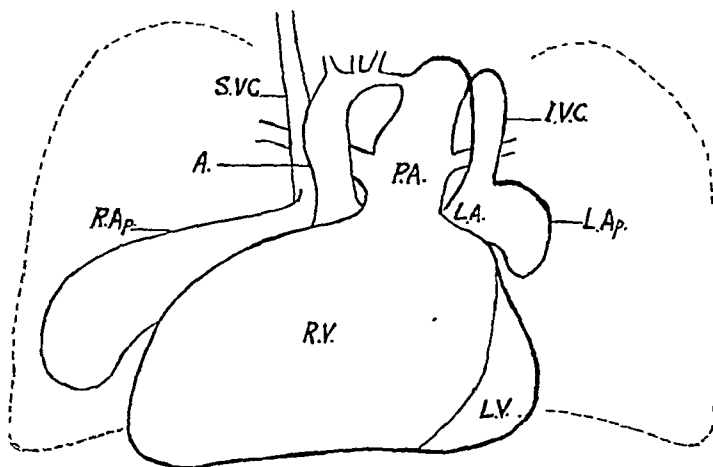


Fig. 2.—A schematic drawing of the heart showing some structures not seen in figure 1. The inferior vena cava enters the left auricle (*L. A.*) at the junction of the latter with its appendage (*L. Ap.*); the other parts are the superior vena cava (*S. V. C.*), the right ventricle (*R. V.*), the left (*L. V.*) and the right auricular appendage (*R. Ap.*).

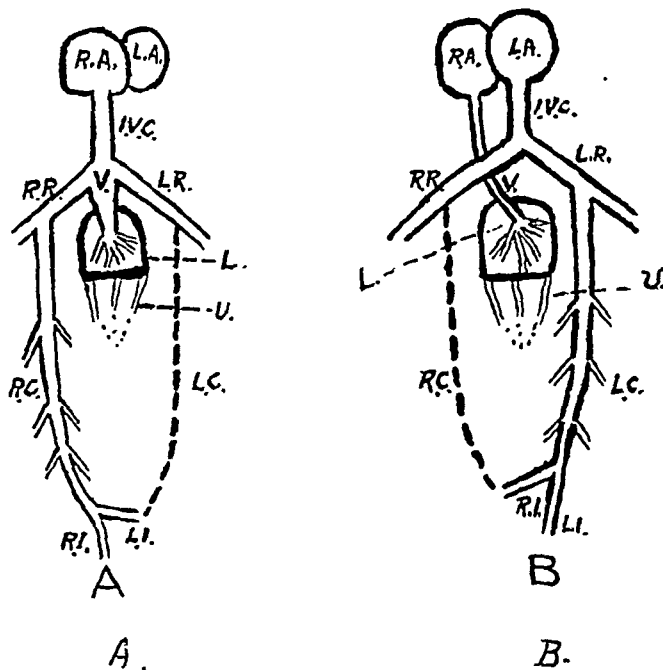


Fig. 3.—*A*, normal development of the inferior vena cava. The posterior right cardinal vein (*R. C.*), its branch the right renal vein (*R. R.*) and the ductus venosus (*V*) join to form the inferior vena cava (*I. V. C.*), which empties into the right auricle (*R. A.*). The posterior left cardinal vein (*L. C.*) disappears and is represented by a dotted line. At its beginning there is survival of a branch (*L. R.*), the left renal vein, and at its very end there is another cross branch (*L. I.*), the future left common iliac vein. *B*, the apparent maldevelopment in our case of a left inferior cava. The right cardinal vein (*R. C.*) is atrophied, only its cross branches *R. R.* and *R. I.* surviving. The left cardinal vein (*L. C.*) is dominant and leads directly into the left auricle (*L. A.*). The hepatic veins (liver, *L*) deriving from the original ductus venosus (*V*) empty directly into the right auricle (*R. A.*). Small veins leading up to the liver are the umbilical and vitelline veins (*U*), undergoing postnatal atrophy.

COMMENT

The persistent ostium primum, while uncommon, has been fully discussed in many reports. The more interesting and really unique anomaly was the inferior vena cava of the left side. In the absence of reports of similar cases we venture an explanation.

The presence of a left inferior vena cava is indicative of an abnormal development in the embryo of the cardinal venous system, particularly of the posterior cardinal veins which drain the lower half of the embryo. Normally, the right lower portions of the posterior cardinal and the subcardinal veins merge, forming a large trunk which in turn unites with the terminal portion of the ductus venosus to form the inferior vena cava. The left posterior veins degenerate and disappear, only their very foremost and lowermost portions surviving to form cross branches with the right cardinal veins, eventually becoming, respectively, the left renal and the left common iliac vein. Evidently, a reverse development occurred in this case, the right posterior cardinal and subcardinal veins disappearing, while the left enlarged and remained as the inferior cava. However, with this survival there appears to have been a further maldevelopment of the venous connection with the heart. The left cardinal vein did not join the terminal end of the ductus venosus; rather it emptied directly into the left auricle. The hepatic venous system remained isolated, draining directly into the right auricle. Unfortunately, the azygos veins were not studied. Such congenital defects must develop very early in embryonic life, probably in the third and fourth weeks (compare Piersol's reconstructions of the human embryo drawn from the His¹ models). Persistence of the ostium primum is also an early developmental defect, possibly occurring simultaneously in this case as part of a widespread maldevelopment of the auriculovenal structure. The presence of the patent ductus arteriosus was distinctly in the nature of a compensatory mechanism, otherwise postnatal life would have been impossible. It is interesting to note that cyanosis was absent for eight days, appearing shortly before death.

SUMMARY

A full term infant, apparently in good health, became cyanotic on the eighth day of life. Marked transverse cardiac enlargement and a loud systolic murmur over the entire precordium were noted. Death occurred on the ninth day. Necropsy revealed a persistent ostium primum, an inferior vena cava on the left side, entering the left auricle, and hepatic veins that emptied directly into the right auricle.

1. Piersol, G. A.: *Human Anatomy*, ed. 5, Philadelphia, J. B. Lippincott Company, 1916, vol. 1, p. 706.

PLASMACYTOMA OF THE UPPER PART OF THE RESPIRATORY PASSAGE

CHARLES M. CAMPBELL JR., M.D., AND FRANCIS C. NEWTON, M.D., BOSTON

The case under consideration is one of plasmacytoma of the upper part of the respiratory passage and is being reported because of the relative rarity of such an occurrence. It is typical in all respects of the majority of the cases presented in the literature. As there are several excellent recent reviews of the previously reported cases, it is felt that a comprehensive review of the literature at this point would be superfluous. Mattick and Thibaudeau¹ published such a review in connection with the report of a case which came under their observation. Two other reviews of the material which are of a comprehensive nature are by Claiborn and Ferris² and Blacklock and Macartney,³ each of which was written in connection with a report of additional cases. Two cases reported by Jackson and his associates⁴ and a case reported by New and Harper,⁵ not included in the aforementioned reviews, bring the total number of reported cases up to 23.

REPORT OF A CASE

A consulting engineer 62 years old entered the Peter Bent Brigham Hospital for the first time on April 6, 1938, because of loss of strength, diarrhea and cough of six weeks' duration. Forty years previously he had an attack of fatigue and malaise associated with palpitation, which was diagnosed as neurasthenia and was improved by rest. Seven years before admission a similar episode associated with diarrhea occurred. Six weeks before admission, feeling "run down," he started on a sea cruise for a rest. Following the resultant sea sickness, for three weeks he was nervous and upset and had loose diarrhea, free from blood or mucus. Shortly thereafter a cold developed with coryza and a productive bronchial cough, accompanied by slight pain in the left side of the chest. During this illness he first became aware of small polypoid masses in back of the uvula. On examination five or six solid red polypi, ranging in size up to nearly 1 cm. in diameter, were seen arising from the posterior tonsillar pillars on either side. There were signs of fluid at the base of the left lung and there was roentgen evidence of pulmonary tuberculosis on that side. Roentgen studies of the bones showed no evidence of metastatic tumor. The diagnosis of tuberculosis was confirmed by injection of the pleural fluid into guinea pigs. The urine showed no Bence Jones protein. The Hinton and Wassermann tests were negative. Other laboratory findings were essentially noncontributory. April 21 the polypi and surrounding areas of mucous

From the departments of pathology and surgery of the Peter Bent Brigham Hospital.

1. Mattick, W. L., and Thibaudeau, A. A.: *Am. J. Cancer* **23**:513, 1935.
2. Claiborn, L. N., and Ferris, M. W.: *Arch. Surg.* **23**:477, 1931.
3. Blacklock, J. W. S., and Macartney, C.: *J. Path. & Bact.* **35**:69, 1932.
4. Jackson, H.; Parker, F., Jr., and Bethea, J. M.: *Am. J. M. Sc.* **181**:169, 1931.
5. New, G. B., and Harper, F. R.: *Arch. Otolaryng.* **16**:50, 1932.

membrane were removed. He was discharged May 7 and went to a sanatorium for treatment of his tuberculosis. Word was received from that institution in July that several small nodules had been removed from the upper part of the respiratory tract and microscopic slides of these lesions were submitted to Dr. S. B. Wolbach for comparison with the original sections.

Pathologic Examination.—At the first operation 6 specimens were received in the pathologic laboratory. These fragments consisted of mucous membrane to which were attached numerous small pedunculated nodules, four of which together, representing the largest group, measured 1.4 by 1 by 0.5 cm. These nodules were of a pinkish yellow color and of a firm rubbery consistency. On sectioning, the cut surface of these nodules was seen to be homogeneous and translucent. There was no evidence that these small tumor masses infiltrated the underlying musculature or the overlying mucosa. A small fragment of one of the nodules was immediately frozen and sectioned. When stained by Giemsa stain, it presented the typical picture of plasmacytoma, with plasma cells densely arranged. The remaining tissue was fixed in part in solution of formaldehyde U. S. P. (1:10) and in part in Zenker fluid with 5 per cent glacial acetic acid. Four sections of material fixed in Zenker fluid were stained with eosin-methylene blue and one with Mallory's phosphotungstic acid-hematoxylin. One of these sections consisted of a thin strip of mucous membrane with a small nodule of sharply circumscribed tumor tissue at each end. These nodules were densely cellular, and under higher magnifying power the cells were seen to be for the most part typical plasma cells, with rough or slightly oval nuclei in which the chromatin was arranged in small dark masses giving the cart wheel or clock face appearance. The finely granular cytoplasm stained light bluish purple. Most of the cells showed paranuclear rarefaction of the cytoplasm, and in these areas the tissue was pink rather than blue. No mitotic figures could be found, but a few cells contained two, rarely more, nuclei. The stroma was very delicate, and in the section stained with phosphotungstic acid-hematoxylin it appeared as a very fine fibrillary network. Around the delicate capillaries the cells were frequently arranged in an acinar-like arrangement, a feature which in the frozen sections gave a confusing resemblance to carcinoma. No definite capsule surrounded the tumor cells, but these did not invade the surrounding tissue. The overlying mucosa was free from evidence of ulceration, and the strip of mucosa between the two nodules was free from abnormal cellular infiltration.

A second section showed a group of four or five small nodules separated by thin strands of moderately dense connective tissue. The tumor nodules resembled those in the preceding section except for a greater number of pyknotic nuclei. This section showed the same absence of infiltration of surrounding tissue by plasma cells.

The third section consisted of a small fragment of tonsil attached to skeletal muscle and connective tissue. No portion of the tumor was included. In the subepithelial connective tissue covering the muscle there was a slight infiltration by lymphocytes, representing a chronic inflammatory process. Among these lymphocytes the plasma cells were more numerous than they usually are in chronically inflamed tissue of this region. Around the follicles of the tonsillar tissue were also seen a few areas of chronic inflammation, and these areas also showed more plasma cells than do the follicles of other chronically inflamed tonsils.

The fourth section stained by this method added no information to that obtained from the three other sections.



A ($\times 65$; eosin-methylene blue) shows the nonencapsulated but moderately distinct edge of the tumor. There is no invasion of the epithelium, which is free from ulceration.

B ($\times 65$; eosin-methylene blue) shows a slight chronic inflammatory change of the subepithelial tissue. The cells in the immediate subepithelial tissue vary from those lying deeper, in that the latter are exclusively plasma cells whereas the former show a heavy admixture of lymphocytes (not shown at this magnification).

C ($\times 680$; eosin-methylene blue) shows that all the cells are typical plasma cells with clock face nuclei. Frequently the paranuclear zone of clear cytoplasm is seen. Note the perivascular arrangement of the cells and the absence of mitotic figures.

Of the material fixed in solution of formaldehyde, one section was impregnated by the method of Levaditi, while the other was stained by the Ziehl-Neelsen method. Careful search of these slides failed to reveal any spirochetes or any acid-fast bacilli.

The tissue submitted from the sanatorium to which the patient had gone for treatment of his tuberculosis was examined by Dr. Wolbach and was reported by him to be morphologically identical to that removed earlier.

COMMENT

Cases of extramedullary plasmacytoma are rare according to reports in the literature. Of such cases, 23 have been reported in which the tumor occurred in the upper part of the respiratory tract; the present case brings the number to 24. The common feature in these 24 cases was the presence of plasma cells densely arranged in discrete nodules. The sex incidence reveals that such tumors occur predominantly in men, 21 of those reported having been found in men and but 3 in women. The majority of the patients were in the fifth and sixth decades of life, but the age of incidence ranges from 20 to 69 years. These generalizations as to the relation of incidence to age and to sex cannot be taken as necessarily valid, because of the small number of cases in the series. Review of the cases showed no other clinical factor to be correlated with the appearance of the tumor. In several of the reported cases the patient was syphilitic, but there is no close correlation between the disease and syphilis, nor can any relation to tuberculosis or any other specific chronic granulomatous disease be discovered.

The pathologic features in the cases reported to date show considerable variation. In 5 of the 24 cases the growth was definitely granulomatous, but these cases must be included in the series because of such features as the density of the plasma cells and the absence or scarcity of other cell types, which serve to differentiate these granulomatous growths from other granulomas and to liken them to the true neoplasm which the other cases represent.

Of those lesions which were definitely neoplastic, only 4 can be considered malignant on the basis of local invasiveness or metastasis to distant organs. The others, it has been assumed, were benign because of the absence of these characteristics. However, in 8 of 16 of the cases, including the present instance, the growth recurred locally after excision. In the remaining 8 cases the patient was not followed long enough after treatment to find whether or not there was recurrence.

In regard to treatment, surgical removal and irradiation are the two methods which have been tried. Radium and roentgen rays together caused marked shrinkage of the tumor in Kaufmann's⁶ case. Claiborn and Ferris followed surgical excision by 33.75 millicurie hours of irradiation, and in their patient no recurrence had taken place in seven months. New and Harper used radium and roentgen rays in combination, which resulted in complete disappearance of the tumor over a period of several months. The most common mode of treatment is excision, but this has not proved entirely satisfactory because of the frequency

6. Kaufmann, E.: *Pathology for Students and Practitioners*, translated by S. P. Reimann, Philadelphia, P. Blakiston's Son & Co., 1929, vol. 1, p. 272.

of recurrence. The series of cases is too small to use as a basis for a dogmatic statement, but the results suggest a combination of thorough surgical removal with subsequent irradiation as the most promising type of treatment.

SUMMARY AND CONCLUSIONS

A case of small plasma cell tumors occurring in the upper part of the respiratory passage of a 62 year old man is reported. These tumors recurred after surgical excision, but the absence of distant metastases and of local invasiveness indicates that the tumors were benign.

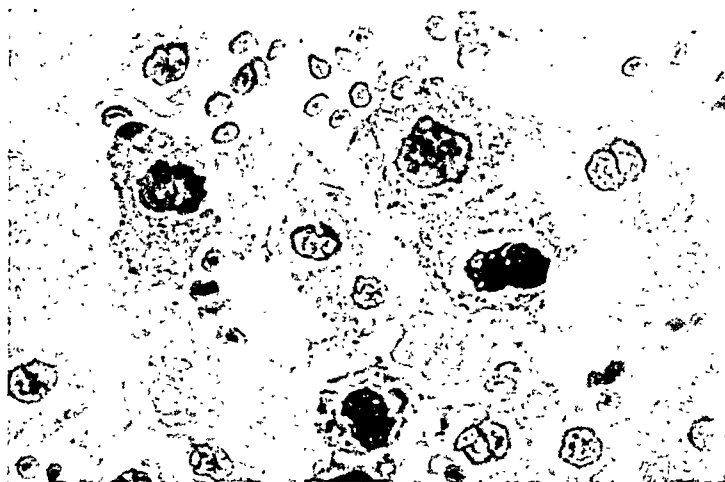
Laboratory Methods and Technical Notes

PONCEAU-FUCHSIN STAIN FOR ANDROGENIC ADRENAL CORTICAL CELLS

A Modified Technic

T. F. FUJIWARA, M.D.,* CLEVELAND

Although the association of hypertrichosis and adiposity with tumor of the adrenal gland was established as long ago as 1756 by W. Cook,¹ it was not until 1933 that a reasonably specific stain differentiating the tumors of androgenic origin from the other types of adrenal cortical tumors was discovered by H. W. C. Vines.² His ponceau-fuchsin stain



Photograph of a colored microdrawing to show the deeply fuchsinophilic character of the so-called androgenic cells.

is claimed to be specific for the cells of androgenic origin, not only for those in the cortical tumors of the adrenal glands giving rise to the androgenital syndrome but also for those in fetal adrenal glands, particularly in the adrenals of the male fetus between the ninth and seventeenth weeks.

A white unmarried woman 23 years old was admitted to the University Hospitals, to the service of Dr. Charles Hudson, with the classic manifestations of the androgenital syndrome. A benign adrenal cortical adenomatous tumor, with the

* Hanna Research Fellow in Pathology.

From the Institute of Pathology, Western Reserve University and University Hospitals.

1. Broster, L. R.: Arch. Surg. **34**:761, 1937.

2. Broster, L. R., and Vines, H. W. C.: The Adrenal Cortex: A Surgical and Pathological Study, London, H. K. Lewis & Co., Ltd., 1933.

flattened adrenal gland attached, was removed surgically by Dr. James Joelson. The entire specimen weighed 135 Gm. The convalescence was uneventful and was followed by marked improvement in the condition of the patient. Sections from the tumor stained with the ponceau-fuchsin stain, by a modified technic, gave a strongly positive reaction. Similarly prepared sections of adrenal tissue from normal males and females of varying ages and of benign and malignant adrenal cortical tumors from patients not manifesting the androgenital syndrome all gave a negative staining reaction. As with the Vines stain, a weak reaction was found in the interstitial cells of the testis, the corpus luteum and the anterior lobe of the pituitary.

PREPARATION OF THE MATERIAL

The material to be examined is fixed for a period of twelve to twenty-four hours in Zenker's fluid containing solution of formaldehyde U. S. P. instead of acetic acid. In order to facilitate proper fixation, the tissue should not be over 3 to 4 mm. in thickness. It should not remain in the fixing fluid for more than twenty-four hours. Best results are obtained when the volume of the fixative exceeds the volume of the tissue at least thirty times. The tissue is then washed in running water for a period of twenty-four hours, after which it is embedded in paraffin and sectioned in the usual manner.

TECHNIC FOR THE PONCEAU-FUCHSIN STAIN

1. Put sections through xylene, alcohol and water in the usual manner.
2. Stain with ponceau-fuchsin five minutes (solution A, 2 parts; solution B, 1 part).

Solution A is made up as follows:

Ponceau de xylidine (Krall).....	1 Gm.
Glacial acetic acid.....	1 cc.
Distilled water	100 cc.

Solution B is made up as follows:

Acid fuchsin	1 Gm.
Glacial acetic acid.....	1 Gm.
Distilled water	100 cc.

3. Rinse in distilled water.
4. Differentiate in a saturated aqueous solution of trinitrophenol (approximately five minutes). One per cent phosphomolybdic acid may be used instead of trinitrophenol.
5. Rinse in distilled water.
6. Dehydrate, clear and mount in salicylate balsam.

In step 4, the weak acid rapidly decolorizes the negative, or nonfuchsinophilic, cells, while the positive, or fuchsinophilic, cells retain the stain for a considerably longer period. In staining an unknown tissue it is advisable to control the degree of differentiation by simultaneously staining a section from a normal adrenal gland or from the anterior lobe of the pituitary or preferably sections from both. The proper differentiation will have been obtained when the control section has been completely decolorized.

General Reviews

VENEREAL LYMPHOGRANULOMA

RIGNEY D'AUNOY, M.D.

AND

EMMERICH VON HAAM, M.D.

NEW ORLEANS

CONTENTS

A comprehensive review of venereal lymphogranuloma has not appeared in American literature. Hugh Stannus' excellent monograph and his supplementary articles appearing in the *Tropical Disease Bulletin* are the only exhaustive treatises on the disease in the English language. Most modern textbooks deal with venereal lymphogranuloma only briefly, and even special monographs on genitourinary infections contain numerous erroneous conceptions regarding the etiologic and pathologic aspects of this disease.

Our intensive study of venereal lymphogranuloma, extending now into its sixth year, afforded us ample opportunity to become familiar with most of the problems presented by the disease and to make many important observations regarding its causal agent, clinical manifestations and pathologic character. We wish to correlate the information previously presented, record the results of our more recent studies and briefly review such important matters as: the history of venereal lymphogranuloma; the geographic distribution and incidence; the clinical manifestations; the pathologic lesions; the biologic characteristics of the causal agent, and the various methods of diagnosis and therapy.

HISTORICAL REVIEW

A historical review of venereal lymphogranuloma is difficult because of the numerous names given the various lesions of the disease before their recognition as manifestations of a distinct clinical entity. To provide proper insight into the history of venereal lymphogranuloma, we deem it best to present the important early contributions dealing with the subject in chronologic order.

Probably the earliest mention of and description of the lesions of venereal lymphogranuloma are found in Wallace's "Treatise on Venereal Diseases," published in 1833. The author gave an accurate word picture

From the Department of Pathology and Bacteriology of the School of Medicine, Louisiana State University, and the Charity Hospital of Louisiana.

of the inguinal bubo with its accompanying constitutional symptoms. Unaware of the true cause of the lesion, he classified it as an "indolent primary syphilitic bubo" and stressed its slow healing and the characteristic involvement of the skin. Eleven years later Desruelles gave an excellent description of 2 cases of vulvar hypertrophy following primary involvement of the inguinal glands. Stannus gave Huguier credit for the first description of this lesion (1848), and Huguier's appellation of it (*esthiomène*) is still used as one of its many designations. Huguier undoubtedly recognized and correctly described for the first time the characteristic induration and discoloration of the affected parts, with the later ulceration and loss of tissue so typical of vulvar hypertrophy. That same year Larsen published the first description of inflammatory stricture of the rectum. All the patients he had observed with stricture of the rectum were females, and he attributed the lesion, which he termed "hyperplastic infiltration of the rectum," to syphilis.

In Europe during the second half of the nineteenth century numerous reports concerning the strumous bubo or *bubon d'emblée* appeared, the lesions in most instances being considered syphilitic. However, Stannus expressed little doubt that "these authors were dealing with cases of lymphogranuloma inguinale." Velpeau described the formation of multiple abscesses in inguinal adenitis, and Reder emphasized that the majority of his patients so affected were males. Nélaton was the first to express the belief that this peculiar inguinal lesion was the manifestation of a new and unknown disease and suggested that the so often accompanying small penile lesions were the sites of entry of its causal agent.

Trousseau described as a not uncommon lesion among the male natives of the islands of Réunion and Mauritius, in the Indian Ocean, unilateral or bilateral inguinal buboes which frequently showed suppuration and often lasted a year or longer. Similar lesions were reported in other colonial territories and were attributed to malaria by some authors (Martin; Ségard). The theory that "climatic influences" of the tropics could cause inflammation of the inguinal glands induced Ruge in 1896 to speak of the lesions as "climatic inflammation of the inguinal glands" and caused Godding to introduce the term "climatic bubo," still found in medical literature. The latter also mentioned superficial penile lesions as possible portals of entry of the infection.

In the United States, similar glandular lesions were described in Philadelphia by Platt, in Baltimore by Winslow and Jones and in Memphis, Tenn., by Armstrong. Klotz in 1890 published a paper, entitled "Strumösen Bubonen," in which he described the classic local and constitutional pictures of the disease from study of some 120 cases encountered over a period of ten years in the German Hospital of New

York. In most of his cases a small penile lesion was present, which he regarded as the point of entrance of the noxious agent producing the bubo. During excision of such a bubo he became infected, large axillary buboes developing.

While the foundation was thus laid for the recognition of "strumous" and "climatic" buboes as manifestations of venereal lymphogranuloma, the causes of esthiomène and of inflammatory stricture of the rectum remained shrouded in darkness. Since Huguier's complete description of esthiomène, numerous reports of similar cases had appeared, syphilis or tuberculosis being ascribed as the cause of the lesions. The principal argument for the syphilitic nature of vulvar elephantiasis was the fact that in the majority of cases there was a history of syphilis, though the complete failure of antisymphilitic treatment tended to disprove the argument. Fournier discussed such tumefaction of the vulva under the name "sclerotic induration of the vulva" in his monograph on syphilis in 1873, and Martineau cited similar cases in his description of syphilitic lesions of the vulva. I. Taylor and MacDonald expressed the belief that the disease was tuberculous and classed it as "lupus of the vulvo-anal region." Bender, in his treatise on tuberculosis of the vulva, recorded many cases of ulcerative lesions of the organ in which the finding of acid-fast organisms was the only basis for the diagnosis. In a critical review of Bender's work, Stannus pointed out that the occurrence of such organisms on the external genitalia is not uncommon, stressing the necessity of properly identifying them as tubercle bacilli by inoculation of animals. Koch in 1896 published 20 cases of "ulcus vulvae chronicum elephantiasicum," emphasizing the sclerotic nature of the disease and the frequent involvement of the perineum and rectum. In his opinion the disease was caused neither by syphilis nor by tuberculosis, a point of view shared also by Pescione. R. W. Taylor attributed tumorous growths on the vulva to simple hyperplasia induced by irritation and trauma, and Verchère expressed the opinion that any venereal disease or tuberculosis could cause elephantiasis of the vulva. Chancroidal, gonorrheal and streptococcic infections were also considered as etiologic factors in the production of vulval hypertrophy (Stannus).

Inflammatory stricture of the rectum caused by the virus of venereal lymphogranuloma presents such a typical clinical picture that descriptions of the lesion can be readily recognized in the medical literature of the nineteenth century even though appearing under various names. Most of the authors of the nineteenth century, basing their conclusions partly on the history of the patients and partly on the histologic picture, held that the lesion was caused by syphilis. Godebert, in his thesis on rectal stricture, stated that he had elicited a history of syphilis in 47 of 67 cases, and Mathews believed that in 60 per cent of his cases of rectal stricture the condition was due to syphilis. Fournier, in his monograph

"The Tertiary Lesions of the Anus and the Rectum," gave a good description of the anorectal syndrome of venereal lymphogranuloma and stressed the cylindric shape of the stricture and the rigid thickening and infiltration of the rectal wall. He named the lesion *syphilome ano rectal*, confessing, however, that his opinion concerning its nature was merely hypothetical. Bryant was inclined to believe that syphilis as a cause of stricture of the rectum was by no means unusual, and only Delbet and Mouchet, Koch and a few others expressed differing opinions. Wallis and also Goodsall and Miles expressed the opinion that ulcerations with superimposed infection were responsible for the development of rectal strictures. Tuberculosis and chancroidal infections were considered as significant etiologic factors by Sourdelle. The lack of recorded cases of esthiomène of the pudenda and rectal stricture among the natives of tropical countries during this period can be explained on the ground that such lesions remained unrecognized or that the patients, mostly prostitutes, shunned medical aid.

From this brief survey it can be seen that all principal lesions of venereal lymphogranuloma had been observed and properly described in the nineteenth century. However, although few contemporaries claimed that there was here a "new and unknown" disease, the true interrelationship of the lesions, the mechanism of infection and the causal agent were unknown.

During the early part of the twentieth century reports on the occurrence of climatic buboes in various colonial possessions became more frequent, as evidenced by the communications of Pigeon and Tanton (290 cases), Priado (50 cases), Müller and Justi (30 cases) and Günther (35 cases). The venereal nature of the infection was pointed out by Rost and others, and intercourse with native women was held responsible for its spread in tropical seaports (Treibly). In 1913 Durand, Nicolas and Favre published the reports of their exhaustive studies on "*les adénites inguinales à foyers purulents intraganglionnaires*." In these, the importance of small herpetiform penile lesions as portals of entry of the causal agent was stressed, and a thorough description of the glandular lesions was given. Because of the histologic resemblance to the lesion encountered in Hodgkin's disease, Durand and his co-workers named the condition "*lymphogranuloma inguinale*," a term which under various modifications is used extensively in modern literature. Recently Sulzberger and Wise proposed the name "*lymphopathia venereum*" for this disease, and Wise prefers to call it "*lymphogranuloma venereum*." In the English literature (Stannus and others) "*inguinal lymphogranuloma*" or "*lymphogranuloma inguinale*" is used. Since the National Conference on Nomenclature of Disease has adopted the term "*venereal lymphogranuloma*" as the official name of the disease, we use it in the present review except in instances in which correct quo-

tation from the literature demands another term. Since this disease is distinctly venereal in character, producing granulomatous lesions principally located in the lymph glands and along the infected lymphatics, we believe that the name adopted here covers the characteristic features of the infection better than any other suggested so far. Certainly the manifestations in the female have little or nothing to do with the inguinal region.

Investigations of this disease were interrupted during the World War but were resumed to some extent after 1920. In 1922 Phylactos published a thesis on the subject, quoting extensively the work of Durand, Nicolas and Favre. In the same year W. H. Hoffmann, as well as Chastang, discussed the possible interrelationship between venereal lymphogranuloma and climatic bubo. Gougerot, on the basis of 20 cases, still maintained that elephantiasis of the vulva was tuberculous, while Stein and Heimann reported a series of similar cases with rectal involvement under the diagnosis of "luetec proctitis and periproctitis." Symonds in 1923 observed 7 cases of what he believed to be "gonorrheal stricture of the rectum" but which according to Stannus were probably venereal lymphogranuloma, and Lockhart-Mummery that same year took a definite stand against the widely publicized opinion that syphilis was the most common cause of stricture of the rectum, contending that it rarely was. Bory in 1928 discussed the importance of *Bacillus subtilis* in the etiology of the Nicolas-Favre disease.

Of tremendous importance to the further development of a more comprehensive understanding of venereal lymphogranuloma as a clinical entity was the discovery by Walter Frei in 1925 of a specific cutaneous reaction resulting from the infection. He showed that an intracutaneous injection of 0.1 cc. of diluted pus taken from the buboes of human venereal lymphogranuloma and sterilized by fractional heating produced in all those affected or who had been affected with the disease marked induration with erythema at the site of injection, reaching its maximum in forty-eight hours. He claimed that when the test was properly performed, the reaction possessed a high degree of specificity for venereal lymphogranuloma. The same year Frei established the connecting link between climatic bubo and venereal lymphogranuloma by demonstrating that an antigen prepared from either lesion gave a positive reaction in a patient afflicted with either condition. Three years later, with Koppel, he obtained positive reactions with Frei antigen in 5 persons with genitoanorectal syndromes. Koppel and many other authors also obtained positive skin reactions in a large number of patients with vulvar elephantiasis and uncomplicated rectal stricture, thus proving the etiologic correlation between these clinical syndromes. Potent antigens were also prepared from the tissues of chronic lesions by de

Gregorio and Murúa, by Nicolas, Favre, Lebeuf and Charpy and by Wiese and Klestadt.

The next achievement in the study of venereal lymphogranuloma was the discovery of the causal agent by Hellerström and Wassén in 1931. After many negative or doubtful reports of successful transmission in animals (Darré and Dumas; Ravaut and co-workers), these Swedish authors succeeded in producing lesions by injecting intracerebrally into certain species of monkeys pus obtained from buboes of patients suffering with venereal lymphogranuloma. From these experimental lesions a filtrable virus was regularly obtained with which the disease could be produced by continued passage in animals. A virus showing identical biologic and physical properties was then isolated from the primary human lesions (Löhe, Rosenfeld, Schlossberger and Krumeich, 1933); from climatic buboes (Findlay, 1933); from tissues taken from chronic lesions—esthiomène and inflammatory stricture of the rectum—(Ravaut and co-workers; Laederich, Levaditi, Mamou and Beauchesne).

The brilliant work of Frei, Hellerström and Wassén, Levaditi and their co-workers cleared the mystery presented by the complexity of lesions caused by this filtrable virus and stimulated further recognition of the disease in all parts of the world. With the nature of its causal agent known and with the help of the diagnostic Frei reaction, nothing should prevent thorough investigation of the mode of infection and spread, the clinical manifestations, the complications, the prevention and the methods of treatment of venereal lymphogranuloma.

GEOGRAPHIC DISTRIBUTION AND INCIDENCE

World Wide Distribution.—Venereal lymphogranuloma in its various manifestations can be found in all quarters of the globe but is more prevalent in tropical, subtropical and temperate climates. On the European continent it is found in probably all of the larger cities, the number of reported cases being steadily on the increase. It is endemic in North and South America, Asia and Australia and along the coasts of Africa.

Hellerström and Wassén in 1933 sent a questionnaire to some 350 clinics for patients with dermatovenereal diseases and collected as a result approximately 1,800 cases of the disease in its various manifestations. Cormia in 1934 estimated the number of reported cases to be about 2,000. During the last two years that number has been at least doubled. We have observed over 600 cases in our venereal diagnostic clinic, and many other series of from 50 to 150 cases have been reported by Navarro Martin, de Gregorio, Advier and Riou, Kalz and Sagher, Pautrier and Weiss, and others during this period. The question arises as to whether this apparent increase in the disease is caused by the

greater ease of its diagnosis and the fact that medical investigators have become more lymphogranuloma conscious or whether there is actually an increase in the number of patients due to rapid spread of the disease. We have shown that in New Orleans at least the first conclusion seems correct, as photographs and descriptions of inguinal lesions contained in old charts from the Charity Hospital at New Orleans leave no doubt but that the disease was observed frequently in New Orleans thirty years ago.

The International Bureau of Public Hygiene of the League of Nations attempted to investigate this problem and sent questionnaires on the subject to the leading venereologists of the European continent. While Jitta of Holland, in response to the questionnaire, did not believe that venereal lymphogranuloma is a public health problem of great importance, venereologists in other countries, especially Germany and Rumania, reported a steady increase of the disease during the past years. The statistics published by Ionesco-Mihaiesti and Longhin are particularly alarming. From 10 cases in 1930 in the city of Bucharest, the capital of Rumania, the number increased over the next three years to 281, and while no cases in females were reported in 1930, 70 were reported in 1933. Reiter recorded 200 cases in Berlin and 100 cases in Breslau, Germany, during the period from 1929 to 1934. Nicolau expressed the opinion that lack of efficient treatment and of prophylactic control of the disease explained this rapid increase, and Koch predicted for the same reasons a higher incidence in the future. Ruge stated that in Hamburg, Germany, during the years 1921 and 1928 the number of cases increased by from 2 to 39 each year, and Frei estimated that between 300 and 400 cases occurred yearly in Berlin. Gougerot and Burnier observed only 9 cases during 1934, in contrast to 389 cases of syphilis. Gibson reported that in the year 1931, 733 inguinal buboes were seen in the English Navy. Many cases of venereal lymphogranuloma have been reported from Italy (Midana and Vercellino; del Vivo), Spain (Bejarano and Gallego Calatayud, Barriola and Maneru, and others), Czechoslovakia (Kalz and Sagher), Russia (Levinson), Sweden (Hellerström) and Norway (Wefring). Perkel and Sourgik stated that the disease occurred along the coast of the Black Sea. Rajam reported cases from India; Tran-Tan-Phat, from Indochina, and Massias, from Cochin China. There are numerous cases reported from China and Japan (Gray and Yieh; Wang and Shen; Kitagawa) and from the Dutch Indies (Bonne and co-workers; Honna and Sasaki). In Africa cases have been observed along the coast of the Belgian Congo (Chesterman), in Tanganyika (Graham), in Algiers, Algeria (Lasnet), in Morocco (Moutot) and in Dakar and surrounding territory (Advier and Riou). The disease is known in South, Central and North America and in Australia (Priado). Coutts, who has studied venereal lymphogranuloma

extensively in Chile, expressed the opinion that it was known to the Romans, Greeks and Arabs and was imported to the New World by the white settlers and slaves. In the Argentine Republic (Zorraquin; Bachmann), Brazil (Crisculo), Venezuela (de Bellard) and Uruguay (Halty), as well as in Mexico (Santos Zetina) and other middle American states, the disease is well known, and cases have been reported from the Bahamas (Kinneard) and the Canaries (Darius Montesino).

In the United States venereal lymphogranuloma has been described in its various manifestations and under various names in such numbers of persons that the disease can no longer be considered rare. Reports have appeared not only from coastal cities and seaports, where there is a possibility of its importation in infected sailors (Wilmoth; Whitmore) but also from many inland cities. From the the eastern section of the United States cases have been reported from New York (Wise; Bloom; Elitzak and Kornblith; and Goldberger and Auslander), from New Jersey (Talbot; Silvers), from Pennsylvania (Martin, several series of case reports from 1933 to 1936; Beacon; Grossman), and from New England states (Howard and Strauss; Giffin). Cases have been reported from the Central states (Amtman and Pilot; Wien and Perlstein; Lash; Reichle and Connor; Dorne and Zakon; Lee and Staley; Dalton). The Southern states are practically all represented in the case reports—Arkansas (Goldstein and Byars), Oklahoma (Allen), Kentucky (Alley), Tennessee (Williams), Florida (A. Brown), Texas (Smith; Lehmann and Pipkin) and Louisiana (D'Aunoy, von Haam and Lichtenstein). In the Middle and Far Western states the disease seems to be rather uncommon, with case reports coming only from Nebraska (Tomlinson and Cameron), California (Templeton and Smith; Diepenbrock and co-workers; Novy) and Washington (Jones). From Canada only a few cases are reported (Desforges; Marin; Bourgouin). In additional cases in Alabama, Georgia, South Carolina, Virginia, Wisconsin and Michigan, although at present unreported, the disease was diagnosed with antigen furnished by us to interested physicians at various medical meetings. This brings the number of states in the United States in which venereal lymphogranuloma has occurred to 27, 11 of these states, or nearly 50 per cent, belonging to the Southern group.

More extensive statistics regarding the frequency of the disease in various large cities of the United States have been published by De Wolf and Van Cleve, S. H. Gray and co-workers, and D'Aunoy and von Haam. The Cleveland investigators published a report in 1932 of a series of 1,010 Frei tests made on patients hospitalized for various conditions; 58 of these gave positive reactions, and in every instance a positive history of venereal lymphogranuloma could be elicited. S. H. Gray and his co-workers, of St. Louis, tested a large number of patients coming to the city venereal clinic and obtained positive reactions in

40 per cent of the colored patients and in 3.4 per cent of the white patients. Using the same test as a routine on all colored patients applying to the outpatient dispensaries of the Charity Hospital of Louisiana at New Orleans, D'Aunoy and von Haam obtained positive results in 17.4 per cent. In most of the cases in which the Frei test was positive a definite history of the disease could be obtained. The same authors were able, during the period from May 1934 to May 1936, to observe 547 clinical cases of the disease in its various manifestations, and in an analysis of the records of 40 cases of elephantiasis of the vulva and 1,285 cases of rectal stricture, von Haam and Lichtenstein came to the conclusion that at least 20 per cent of the cases of elephantiasis and most of the cases of inflammatory stricture of the rectum observed in New Orleans from 1911 to 1935 could justifiably be suspected as cases of venereal lymphogranuloma.

From this brief résumé of the geographic distribution of venereal lymphogranuloma it can be concluded that the disease is an endemic infection of countries with warm or moderate climates. Regions of high altitude, such as the Rocky Mountain areas of the United States, the European Alps and the highlands of Tibet, do not have many cases, while low lands with considerable humidity, such as the Mississippi Valley, the coastal marshlands of Louisiana and the area about the Black Sea, seem favorable to the spread of the disease.

Racial Incidence.—It is generally stated by authors who have had opportunity to observe large numbers of cases of venereal lymphogranuloma that the Negro race seems more prone to contract the disease than the white race. Observations in North America are especially valuable in this respect because of the racial mixture of the patients frequenting the free clinics of the larger American cities. In Philadelphia, with a Negro population of practically 11 per cent, Bacon observed 31 cases in white persons and 118 in Negroes. In Indianapolis, according to Dalton, inguinal lymphogranuloma occurs as frequently in white persons as in Negroes. In Cincinnati 11 of the 16 cases of inflammatory stricture of the rectum observed by Lee and Staley concerned Negroes. In Cleveland De Wolf and Van Cleve saw twice as many Negro men as white men with inguinal buboes due to venereal lymphogranuloma, and Gray and his co-workers obtained positive Frei reactions in only 3.4 per cent of their white patients, while 40 per cent of their Negro patients had positive reactions. Our records demonstrate still better this racial difference in the incidence of venereal lymphogranuloma. Of our 547 patients, only 24 belonged to the white race. A satisfactory explanation of this peculiar racial incidence of venereal lymphogranuloma cannot be given at present. C. F. Martin discusses the possibility of a "fibroplastic diathesis" (Rosser) in the Negro race.

Stannus expressed the opinion that the manner of living and the loose moral code of Negroes may account for the preponderance of the disease in their race. Although racial constitution is no doubt an important factor in the distribution of many diseases, we are inclined to agree with Stannus that living conditions and loose social conditions are responsible for the wide occurrence of venereal lymphogranuloma in Negroes.

Sex Incidence.—As long as the various clinical manifestations and pathologic lesions of venereal lymphogranuloma were not fully known, the disease was believed to be restricted to the male sex (Hanschell; de Bellard). Only when it was proved that esthiomène of the vulva and inflammatory stricture of the rectum were manifestations of venereal lymphogranuloma was it realized that the disease occurred in women, although with different clinical and pathologic pictures. No doubt there is a preponderance of the disease in males, but this preponderance is not as marked as was maintained in the older literature. Gray and co-workers reported the same incidence of positive Frei reactions in men as in women. In our series of 547 patients 194 were females and 353 were males. In a series of routine Frei tests on 960 females and 509 males, we obtained 118 positive reactions in men and 79 in women. These figures perhaps represent more accurately the true sex incidence of the disease than do figures based on a single clinical manifestation of the disease (De Wolf and Van Cleve; Hellerström; Phylactos, and others). The explanation for this sex difference is purely hypothetic. Pautrier suggested that the virus of venereal lymphogranuloma may exist in the vagina as a harmless saprophyte. Coutts and Banderas Bianchi shared this opinion, although there is no experimental proof therefor. Much more plausible seems the explanation of Schulmann that in women the disease during the acute stage is mild and passes unnoticed or that the lesions are usually deep seated, hidden in the vagina (Naumann) and not readily recognized. L. A. Gray stressed the importance of chronic urethritis in women as a manifestation of venereal lymphogranuloma. Chevallier and Bernard observed a female patient in whom chronic periurethral edema was the only clinical evidence of infection. That the disease is transmitted from male to female and vice versa is proved in numerous so-called partner cases in which the history of infection can be traced to coitus or sexual perversity (Lévy-Franckel and Temerson; Chevallier and Moline; Nicolas and Lebeuf; Juvin; Hoffmann; Lépinay and Grévin). We are of the opinion that as knowledge of the various clinical forms of the disease progresses the difference in its sex incidence will be greatly reduced.

Age Incidence.—As with other venereal diseases, the largest incidence of venereal lymphogranuloma is in persons who are between the

twentieth and fortieth years of age—the period of greatest sexual activity. Seventy of our patients were between 14 and 20 years of age; 11 were over 50 years of age. Cases in children have been reported by Weiss and Cain, Chevallier and co-workers, Elitzak and Kornblith, and Luján and Rotter. In the case reported by Luján and Rotter, mere contact with infected adults was responsible for the infection. Since women suffer more from the chronic manifestations of the disease (esthiomène and inflammatory stricture of the rectum), female patients are of a higher average age than are male patients. All of our patients over 40 years of age were women.

The question whether venereal lymphogranuloma can be transmitted from parent to offspring has been discussed by Dick, who reported a case in which the apparently well child of infected parents had a positive Frei reaction and another case in which the apparently healthy newborn infant of a mother with rectal stricture had a positive Frei reaction. Since the diagnostic value of the Frei test at birth and in early childhood has been doubted because of the tenderness of the skin, we do not believe that Dick's findings are sufficient proof for his conclusions.

Occupational Incidence.—Venereal lymphogranuloma is reputed to be a disease of seaports, of the slum districts of larger cities, and of brothels and their devotees. Undoubtedly the afflicted patients belong to low social classes. We have observed but a single case in a person of high social standing. The female patients are usually prostitutes, a class in which an incidence of as high as 10 to 15 per cent is reported. Schulmann and also Bejarano and Gallego Calatayud expressed the belief that at least 10 per cent of all prostitutes are infected. The largest group of white male patients is made up of sailors; so the disease is well known to naval medical officers. Philipps, Whitmore, Treibly and Wilmoth have described cases in the American Navy. Gibson stressed the importance of the disease for the English Navy, especially for the far eastern contingents. Hanschell as well as Müller and Justi noted that the engine room crews, exposed to hot and humid atmospheres, are more susceptible to the infection. C. F. Martin issued a stern warning concerning the rapid and uncontrolled increase of the disease among persons of the lower strata of society, stressing the financial burden which treatment of the disease in free clinics will bring to communities. He expressed a pessimistic outlook regarding the disease. Two years after recognition of venereal lymphogranuloma in New Orleans, it became necessary to open a special clinic for it. Here, from 10 to 12 new patients are seen each day. Additionally, the public hospital wards are filled with patients suffering hopelessly with inflammatory stricture of the rectum. Venereal lymphogranuloma is a disease which in our opinion threatens to become a grave public health problem in the United States, especially in its southern portion.

CLINICAL MANIFESTATIONS

Few diseases present such a variety of clinical symptoms and pathologic lesions as does venereal lymphogranuloma. Many of these seem so little related to each other that some authors still hesitate to attribute them to the action of a single etiologic agent. With the aid of Frei's intradermal reaction, we now believe, recognition of at least the principal manifestations of this venereal disease is possible. For better analysis of the symptom complex of venereal lymphogranuloma, it is deemed convenient to consider separately such symptoms as are related to the circumscribed genital and extragenital lesions and such as may be considered evidences of general invasion. Little is known concerning the evolution of the disease as a systemic complex, although much evidence can be adduced in favor of such an invasion actually occurring. In our clinical studies we were much impressed by the fact that the disease frequently consists not only of local manifestations but also of rather general systemic reactions.

Primary Lesions.—Various types of primary lesions may be encountered. Sézary and Drain described five types. The syphiloid, nodular and infiltrated types of primary lesions are seen rarely. They have been reported by a number of authors (Sézary, Bolgert and Joseph; Sézary and Perrault; Bory; Ravaut and Scheikevitch; Nicolas, Lebeuf and Rousset, and others). The most common type is a small herpes-like eruption, usually located near the coronal sulcus, neither tender nor suppurating and showing a tendency toward spontaneous healing. In the majority of cases this lesion is completely overlooked by the patient and has usually disappeared when he consults a doctor. In our series, 47 lesions of this type were seen in men and 2 in women; in both of the latter cases the lesion was located on the inside of the labia minora. The so-called lymphogranulomatous chancre is inclined to persist for some time, shows a rather deeply ulcerated area and is associated with more or less distinct lymphangitis, which can usually be traced to the swollen glands at the root of the penis. Sometimes, especially in patients with long or narrow prepuces, a nonspecific balanitis is present.

Indeed interesting are the reports of a primary lesion within the male or the female urethra with or without the formation of a small, well defined ulceration. A lesion at this site is supposedly more frequent in females. Gray reported 25 cases in which such a lesion resulted in urethral stricture. Curth, Frei and co-workers, Kalz, Polak and Bezecny have described similar cases, and Stannus considered a possible relationship between such lesions and the nonspecific types of urethritis described by Waelsch and more recently by Hissard and Husson.

Extragenital primary lesions have been ascribed to infection contracted while handling patients (Klotz; Phylactos; Hellerström; Homma and Chaglassian) or as a result of sexual perversities, such as cunnilingus or coitus in ano (Curth; Bezecky and Sagher; Buschke and Curth; Bloom; Wehrbein, Buschke, Boss and Vassarhelyi). Coutts described 12 cases in which there were diffuse swelling and thickening of the tongue—glossitis marginata—which he considered as a lesion of venereal lymphogranuloma contracted through cunnilingus.

Inguinal Bubo.—This must be regarded as the principal manifestation of the acute stage of venereal lymphogranuloma. It begins as a firm hard mass, not very painful and usually involving several groups of lymph nodes (von Haam and Lichtenstein). Within one or two weeks the glandular mass becomes attached to the skin and subcutaneous tissue, and fluctuation can be noted. It is at this stage that the patient complains of severe pain in the groin. Usually the skin now takes on a characteristic livid discoloration, which has led to the popular term "blue balls," and its shiny appearance predicts threatening rupture of the mass. Some buboes, however, never reach this stage, and involution of the firm masses without suppuration may be observed—the so-called abortive form of venereal lymphogranuloma. However, once the stage of fluctuation is established, suppuration with the formation of sinuses in the skin is unavoidable. Perforation of the bubo through the skin usually relieves the pain, and many patients carry such fistulous sinuses for many months without complaining of much discomfort. As a rule, numerous sinuses are formed, giving the inguinal region the appearance of the mouthpiece of a watering pot. This has caused Fiessinger to introduce the name "poradenitis." Healing of such a fistulous mass is usually slow, and the sinuses continue to drain pus for months, even years. The scars which later form in the inguinal region are callous and contracted and extremely characteristic of the disease.

The marked difference in the frequency of inguinal buboes in male and female patients suffering from the disease is sufficiently explained by the different anatomic distribution of the lymphatics in the sexes (Stannus). The lymphatics of the penis, the most common site of the primary lesion in males, drain to the superficial and deep inguinal glands; those of the deeper parts of the vagina or the cervix, the area which is probably the most common site of the primary infection in females, drain to the external iliac, retrocruial, hypogastric, pararectal and parasacral glands. Whether glandular disease similar to that observed in males occurs in females in the deeper seated glands, which are unavailable for inspection, or whether the acute infection in females takes a different course, is pure speculation at present. In our series, 278 inguinal buboes were observed in males and only 31 in females. These

figures coincide with those reported by other observers (Reiter; Gottlieb; Frei and Hoffmann). The majority of our patients (187 men and 20 women) had unilateral glandular buboes, a finding stressed by Ruge and Hellerström. The femoral and iliac glands are but rarely affected (Kitchevatz and Alcalay). If the primary lesion is extragenital, the regional glands undergo changes similar to those seen in inguinal bubo (Klotz; Coutts; Curth). Chevallier and Moline reported a case of conjugal infection between husband and wife, in which the woman had a swollen palatal gland, a feature seen otherwise only in von Mikulicz' disease. General adenopathy has been reported as a consequence of infection with the virus (Chevallier and Barreau) but is considered rare by Stannus. The principal clinical symptoms produced by these buboes are: pain, complained of in 52.9 per cent of our series; local tenderness, noted in 83.5 per cent; suppuration, present in 39.4 per cent.

Coutt's attempt to divide the disease, according to lymph gland involvement, into two different entities caused by virus A and virus B has met with severe criticism by Stannus. On the basis of our experience we are not able to accept Coutt's classification. Lymphangitis may accompany the bubo and has been reported by Coutts (thrombolympangitis of the penis) and by Nicolau and Banciu. Several times we have observed swollen dorsal lymphatics of the penis. One case was noted in which suppuration was present in three distinct sections of such a lymph vessel. The disappearance of the inguinal bubo usually marks the end of the disease in the male, and in the majority of cases no serious sequelae result.

In contrast to the course of lymphogranuloma in males, in females the manifestations of the disease are frequently chronic—*esthiomène* and inflammatory stricture of the rectum.

Esthiomène; Elephantiasis of the Pudenda; Genitoanorectal Syndrome.—Since the report of Frei and Koppel in 1928 and of Koppel in 1929, numerous authors have proved beyond doubt that venereal lymphogranuloma is the cause of hypertrophic ulceration of the pudenda in most of the cases. Hellerström in 1934 listed over 200 cases of *esthiomène*, reported by 22 authors; 97.5 per cent of the patients had positive Frei reactions. Although more common in females, hypertrophic lesions can be observed also in males, involving the penis and the scrotal sac. In our series we have observed hypertrophic ulceration of the pudenda in 56 patients, 3 of whom were of the male sex. In 27 of the females the ulcerative process was more outstanding, while in 26 elephantiasis of the parts involved was dominant. There was no distinct correlation between the ulcerative and the hypertrophic changes as regards either location or duration. In some cases, the mucous membrane of the hypertrophied labia presented rather superficial and irregular ulcers; these were tender to touch and resisted all treatment. From 13 females a his-

tory of previous inguinal bubo could be elicited. The hypertrophic type of lesion shows at first only thickening of the affected parts, resembling chronic edema. The mucous membrane feels leathery and dry. With increase in size of the tissues, discoloration of the mucous membrane over the affected parts can be noted, with secretion of a thin cloudy fluid. Sometimes rather extensive superficial ulcerations appear and present all the usual signs of an acute pyogenic infection. As regards the distribution of the lesions and their extension, we may state from our observations that no definite rules are followed, the disease following a different course in each case, a fact which makes the clinical recognition of the condition difficult. In contrast to the inguinal bubo, however, we observed that involvement of parts of the pudenda was more often bilateral, with one side usually more severely affected. Five cases of isolated hypertrophy of the clitoris came under our observation.

Our 3 cases of *esthiomène* in males included, respectively, elephantiasis of the entire scrotum, elephantiasis of the left part of the scrotum and elephantiasis of the penis. In the case in which the condition was unilateral, complete extirpation of the glands of the affected side was followed for many months by a fistulous process within the wound before enlargement of the left side of the scrotum was noted. Similar cases have been described by others (Nicolau; Coutts and Martini Herrera; Navarro Martin; Louste, Cailliau and Schwartz). An interesting case in which the *esthiomène* apparently originated as a granulating tumor of the urethra has been described by Bezecky.

Secondary complications often bring serious consequences to patients suffering from hypertrophic lesions. Pyogenic infection and attacks of localized erysipelas are frequently observed in the tissues which have been deeply altered by the pathologic process. Carcinoma may develop on the basis of *esthiomène*, as in the cases observed by Bernstein and Philipp.

The principal complaints of patients suffering from hypertrophic lesions are local tenderness and an abundant secretion of exudate which soils the clothing. In some cases, however, no disagreeable consequences are felt, and the growth is permitted to reach fantastic dimensions.

The association of *esthiomène* with lesions involving the perineum, anus and rectum is reported with varying frequency in the literature. We have observed only 10 cases of this so-called genitoanorectal syndrome (Jersild); 7 of the patients were women and 3 were men. The exact evolution of this syndrome is still disputed, and while some authors believe that it is the result of retrograde spreading of specific lymphangitis (Stannus), Frei expressed the view that it represents actual infection of the subcutaneous tissue by the virus. Feilchenfeld maintained that in all cases of genitoanorectal lesions the primary lesion was in or around the anus, and Coutts claimed pederasty as its determining cause.

We have elicited a definite history of coitus in ano from 1 male and 4 female patients so affected. Kiefer observed the development of this syndrome after a furuncle-like primary lesion in the coccygeal region, and Ravaut, Levaditi and Maisler stressed its contagious nature by citing a partner case. Gougerot and Carteaud described the early appearance of the lesion as that of a small circumscribed nodule which soon becomes fistulous. Several such nodules appear, and the skin between them becomes edematous and hypertrophic. When fully developed, these lesions, as observed by us, show marked fibrosis and moderate hypertrophy of the perineum with numerous small sinuses and fistulas, extensive ulceration and always characteristic tags about the anus.

Inflammatory Stricture of the Rectum.—This lesion is without doubt the most serious manifestation of venereal lymphogranuloma. We have collected 97 cases. Of the patients, 90 were women and 7 were men; 5 (4 women and 1 man) were white, while the others were Negroes. Fifty-eight of our cases have been thoroughly analyzed and described (Lichtenstein). The clinical picture of the condition is rather uniform, and the history and the results of physical examination usually allow a correct diagnosis. The complaints are those of low grade obstruction with infection of the rectum and associated lesions of the anus and perirectal tissue. In prolongation of the condition cachexia, anemia and severe pellagra are noted. The rectal examination reveals in most instances an annular stricture 4 to 6 cm. above the anus, where it usually can be reached easily with the examining finger. The induration of the rectal wall is varying and in advanced cases produces such constriction of the lumen that the index finger will not be admitted. Thickening of the rectovaginal septum has been found in a great number of our cases and can be stressed as a valuable differential diagnostic sign. Below the stricture the mucous membrane of the rectum generally shows ulcerative and granulomatous proctitis, which makes examination extremely painful. The anal orifice is usually surrounded by large cauliflower-like excrescences. The most dreaded complication is the rectovaginal fistula, which was present in 14 per cent of our cases and which usually recurred after surgical repair. The fistulas which appear on the perineum sometimes heal spontaneously, only to recur as the disease progresses. C. F. Martin described the appearance of the diseased perirectal tissue and perineum as "rat bitten" and stressed the unyieldability and consequent undilatability of the stricture. He believed this condition to be worse than any manifestation of syphilis, "ranking very close to malignancy in its therapeutic aspects." Alley subdivided inflammatory stricture of the rectum into simple anorectitis, proctitis obliterans and fibrous stricture of the rectum. S  n  que described simple rectal strictures and strictures complicated with fistulas, with elephantiasis and

with pelvic cellulitis. Of the 11 patients dying with rectal stricture in the Charity Hospital at New Orleans during 1934-1935, 2 died of peritonitis caused by rupture of the stricture during dilatation and 4 following colostomy. The other 5 patients died with extreme cachexia, prostration and large decubital ulcers. Fifteen per cent of our patients with advanced stricture of the rectum showed typical pellagra. We concur in Martin's pessimistic prognosis regarding this manifestation of venereal lymphogranuloma.

Constitutional Symptoms and Remote Lesions.—More or less severe constitutional symptoms are rather characteristic of the acute stage of venereal lymphogranuloma, and they must be considered as important factors in the differential diagnosis. Rise in temperature occurred in over 50 per cent of our cases and has been stressed by others as a noteworthy sign in the differential diagnosis between venereal lymphogranuloma and soft chancre (Löhe and Blümmers; Guttentag; Buschke, Boss and Vasarhelyi). It usually precedes the appearance of the bubo and is accompanied by anorexia and prostration. Irregular temperature with chills indicates severe generalized virus infection. Headache was noted in over one third of our cases. Dizziness and slight nausea were usually present, as a rule forcing the patients to bed. Meningeal reaction with stiffness of the neck was observed by Nicolas and Lebeuf and by us, while Chevallier and Bernard reported what was possibly chronic meningitis in a woman suffering from the disease. Insomnia was stressed by Tran-Tan-Phat. Anorexia with occasional vomiting was present in 11 per cent of our cases, and Giacardy and Guttentag reported cases in which there was a resemblance to acute enteric infections. Loss of weight was prominent in 25 per cent of our series. One patient experienced a weight decrease of 30 pounds (13.5 Kg.) during the first week of the disease. Backaches were rather characteristic in our female patients, though rarely seen in men; this, we believe, can be taken as an indication that infection of the deeper pelvic glands is frequent in females. It is, as well, a constant symptom in patients suffering from stricture of the rectum and is sometimes associated with acute pelvic symptoms (Franchi). Various authors have recorded changes in the blood picture, although these are not believed to be characteristic (Nicolau; Stannus). Ravaut and Cachera found an increase of the mononuclear cells up to 17 per cent, and Ruge observed an increase in the total white cell count. The observation of mononucleosis was confirmed by others (Bernucci; Kristjansen; Gay Prieto). Chevallier and Bernard and Coutts and Banderas Bianchi found eosinophilia varying from 2 to 23 per cent. Coutts found that anemia is constantly present to a marked degree in what he terms the "second stage of the disease," an observation not made by Ruge or by us. Serologic changes have led to the introduction of various diagnostic methods, which will

be discussed in another chapter. The spinal fluid has been examined especially with reference to cephalalgia (Ravaut and Scheikevitch; Midana and Vercellino; Kitagawa). The hypertension of the spinal fluid observed by Kitagawa in 30 cases could not be found by Chaigneau, while the findings of Midana and Vercellino were refuted by Ravaut and Scheikevitch. Our examination of the spinal fluid in numerous cases did not show any deviation from the norm.

Remote lesions ascribed to venereal lymphogranuloma are being observed with increasing frequency, but caution in their evaluation is necessary in order to avoid erroneous conclusions. Short-lasting attacks of polyarthritides have been observed by a number of authors (Durand, Nicolas and Favre; Löhe and Blümmers), while others have reported true arthritis with exudative and chronic changes in the joint (Frauchiger; Caciro Carrasco; Koppel; H. Hoffmann; Gottlieb, and others). Coutts mentioned the similarity of the disease in such a form to gonorrheal arthritis and prepared a potent antigen from the exudate occurring in the joints. Severe involvement of joints, with sepsis was noted by Buschke, Boss and Vasarhelyi and by Reichle and Connor. Cutaneous lesions produced by the virus can be divided into two groups: general exanthems and local skin changes around or close to the genital lesions. Among the first, two types of erythema nodosum, multiforme and exudativum, have been described by numerous authors (Hellerström; Kleeberg; Frei and Hoffmann; Koppel; Chevallier and Bernard; Cuesta de la Almonacid; Gans; Kitchevatz; Buschke; Pinard and Fiehrer; Löhe and Blümmers). Representing the second group, small pustular or nodular lesions localized in the genital area have been observed (Pinard and Fiehrer; Cuesta de la Almonacid; Lévy-Franckel and Temerson; Sézary and Bardin; Chevallier and Bernard; D'Aunoy and von Haam). Stomatitis was observed by Gottlieb and by Löhe and Blümmers in their cases; conjunctivitis and episcleritis, by Gottlieb, Hellerström and Koppel as well as by us in 2 instances. Eyeground changes—peripapillary edema of the retina and tortuosity of the vessels—were described by Kitagawa; the observation was confirmed by Coutts and considered by him as "highly specific" for the disease. Clinical suggestions that lesions occur also in the spleen and liver (Chevallier, Moricard and Lévy-Bruhl; Naumann; Pardo-Castello) and lungs (Hansmann) still require adequate confirmation.

It cannot be denied that the presence of constitutional symptoms and the occurrence of remote lesions during the course of venereal lymphogranuloma suggest a generalized systemic infection. However, considering the great rarity with which these remote lesions are encountered as contrasted with the frequency of the manifestations localized to the genital area, we believe that it can be assumed that in most instances disseminated virus is soon destroyed.

PATHOLOGY

In contrast to the rapidly increasing literature dealing with the clinical and statistical aspects of venereal lymphogranuloma, very little has been published recently concerning the pathologic changes initiated by the disease. The reason for this is twofold: first, most of the lesions are evanescent, and it is usually difficult to obtain biopsy material; second, many observers considered the histologic descriptions given before discovery of the Frei test as sufficient and needing no further elaboration. The material at our disposal has allowed us to study practically all of the pathologic changes described by various authors, and our numerous transmission experiments furnished rich material for the study of lesions produced by the virus in most species of animals. We shall now compare our observations with those recorded in the literature and attempt to trace the evolution of the disease from its primary lesion to its final stage.

Human Lesions.—We studied histologically two types of primary lesions—the lymphogranulomatous chancre and the herpetiform preputial lesion. The chancroidal form has a histologic appearance similar to that of a soft chancre of the Ducrey type. It consists essentially of necrosis of the epithelium and underlying connective tissue with abundant circumferential epithelial proliferation (von Haam). In the zone removed from the immediate region of necrosis and ulceration the lymph vessels are markedly dilated and filled with large endothelial cells. The herpetiform lesion is characterized by hyperplasia of the stratum granulosum with marked intraepithelial edema and dense infiltration of the entire rete Malpighii by small and large round cells and neutrophilic leukocytes.

The inguinal buboes have been so splendidly described by Durand, Nicolas and Favre that little can be added. The excised inguinal masses usually consist of a number of enlarged glands held firmly together by plastic periadenitis. In the early stage of the disease only a grayish, sometimes slightly hemorrhagic swelling of the glands is noted, but as the process advances the glandular tissue takes on a yellowish gelatinous appearance interspersed with small grayish white pinpoint areas. These are miliary abscesses. At this stage the periglandular tissue is usually markedly hemorrhagic and very edematous. The small focal abscesses enlarge, many become confluent, and finally pus breaks through the capsule of the gland, reaching the surface through numerous sinuses. If the patient is not treated surgically, these sinuses continue to form until the overlying skin appears as a sieve. The process has now reached its point of culmination and, with slow formation of a fibrous scar, healing follows, generally interrupted by frequent flare-ups resulting from pyogenic infections. Histologically the inguinal bubo of venereal

lymphogranuloma is especially characterized by proliferation of the endothelial cells lining the lymph spaces of the glands. Massing of large mononuclear cells occurs in the form of more or less circumscribed nodules—the *gommès lymphogranulomatosiques* of French authors. In some cases the disease remains arrested at this stage, with no actual destruction of tissue. In such cases firm, often indolent glands remain palpable for a long time, slowly undergoing resolution (*formes frustes*). In the majority of cases, however, invasion of the endothelial nodules by polymorphonuclear leukocytes takes place; the centers of the nodules become necrotic, and small abscesses surrounded by rather densely packed endothelial cells result. These abscesses usually assume a triangular or quadrangular shape (stellate abscesses) and diagnostically are quite characteristic. At the same time, fibrosis begins from the capsule of the node, a markedly vascular granulation tissue invaded by numerous plasma cells completely destroying the normal histologic structure of the gland. In chronic conditions no characteristic histologic glandular lesion can be noted. In the pus from inguinal buboes, as well as in the necrotic débris of the small stellate abscesses, small intracellular bodies were described by Gamna and Favre, who suggested that they represent inclusion bodies. Fischl, Todd and Bory confirmed the observation of such intracytoplasmic chromatophil bodies but expressed the opinion that they were phagocytosed cellular débris. Findlay, on the basis of careful study, classified them as nucleolar extrusions of the type frequently noted in other pathologic conditions. They are not identical with the small granulocorpuscles described by Miyagawa and his co-workers as corpuscular forms of the virus. We have seen similar structures, stained very irregularly with hematoxylin and eosin, in smears of pus and believe them to be nuclear débris resulting from necrobiosis.

Esthionène and Inflammatory Stricture of the Rectum.—In their basic pathogenesis these chronic manifestations of venereal lymphogranuloma are identical, even though they occur in different anatomic structures; which develops depends largely on the location of the primarily infected lymph glands. Involvement of the inguinal, presymphysial or crural lymph nodes will affect the scrotum, penis or vulva, while infection of the anorectal, deep iliac and presacral glands will cause involvement of the perineum and of the lower part of the rectum. The essential pathologic process in both conditions is thrombendolymphangitis and perilymphangitis with a tendency to spread from the infected glands into the surrounding tissue. The changes in the lymph vessels are probably caused by direct action of the virus, as indicated by the experiments of Ravaut, Levaditi, Lambling and Cachera and of Löhe and Rosenfeld, and are not merely the effect of lymph stasis as Frei

believed. This chronic progressive lymphangitis accompanied by chronic edema and sclerosing fibrosis of the subcutaneous and submucous tissues results in induration and enlargement of the affected parts. The covering epithelium finally suffers from insufficient circulation, and ulceration occurs. These changes are observed earlier in the rectum, where the mucous membrane is more sensitive to changes in the submucous tissues, than in the skin of the perineum and genitalia. Pyogens invading such ulcerated areas play havoc in the altered tissue structures and establish foci of infection, ultimately responsible for the fast downhill course of patients afflicted with these lesions. The histologic picture of these chronic lesions is not characteristic and does not permit accurate diagnosis as conclusively as does the picture encountered in the acute bubo. Briefly summarized, the essential changes in rectal stricture are: destruction and ulceration of the mucosa; infiltration of the muscular layer by miliary accumulations of lymphocytes and plasma cells; dilatation of the lymphatics with perilymphatic infiltrations; thrombendo-lymphangitis (Barthels and Biberstein; Lichtenstein). Often the blood vessels show some endarteritic changes with narrowing of their lumens, which, however, is never as marked as in syphilis. The presence of *specific granulation tissue comparable to the endothelial nodules in the lymph glands* has been described in the chronic lesions by Gougerot and Carteaud, who observed *veritable gommès de Nicolas Favre* in very early lesions of elephantiasis of the genitalia and anus. These consisted of well circumscribed accumulations of neutrophilic leukocytes, eosinophils, mononuclear cells, plasma cells and epithelioid cells, showing at their centers homogeneous pink-staining material containing pyknotic and destroyed leukocytes and surrounded by dilated blood and lymph vessels, the latter usually filled with plasma cells. Barthels and Biberstein observed similar lesions in the ampulla of a rectum extirpated because of inflammatory stricture but thought that the gumma-like masses and small stellate abscesses were localized in the small lymph nodes and did not occur in the connective tissue. We have observed a case of elephantiasis of the vulva with numerous similar small gummas located beneath the mucous membrane. In these microscopic examination revealed large numbers of giant cells as noted by Babès in a similar case. Syphilis could be excluded. As frequent sequelae of rectal stricture, anal tags must be mentioned. Histologically these are composed entirely of dilated lymph vessels with perilymphatic inflammation. Lichtenstein suggested the name "lymphorroids" for these formations, which clinically are usually diagnosed as hemorrhoids. The sclerosing process extends far into the periproctal tissue, and the rectum has the appearance of being encased in cement. Numerous adhesions fix the lower part of the sigmoid and the rectum to the wall of the pelvis and to the neighboring organs, and in some cases a picture resembling that in chronic pelvic

inflammatory disease is produced. The histologic changes in the ulcerated mucous membrane are essentially those encountered in the ulcerations occurring in esthiomène of the pudenda. A primary disease of the rectal mucosa caused by the virus of venereal lymphogranuloma, so-called anoproctitis, which may give rise to rectal stricture, has been suggested by Coutts, who ascribed pederasty as its determining cause.

Extragenital Lesions.—Reports on the pathogenesis of extragenital lesions are rare, and much information is needed in order to draw accurate conclusions regarding widespread dissemination of the virus during the course of the disease. Gans described the histologic picture of a papular erythema observed in a patient with venereal lymphogranuloma. The lesion was strictly localized and consisted of only 10 to 15 papillae. The infiltrative leukocytic process was confined principally to areas around the blood vessels, in which the endothelial linings were swollen and desquamated. We observed a fistulous subacute epididymitis in a man with a strongly positive Frei reaction. The testis and epididymis were seeded with small stellate abscesses from which sinuses led to the skin of the scrotum. Microscopic examination showed a typical endothelial reaction with central necrosis and suppuration similar to that seen in lymph glands. D'Aunoy and Schenken observed venereal lymphogranuloma of the fallopian tube in a 20 year old Negress, who gave a history of pelvic pains with menstrual disturbance of six weeks' duration. A clinical diagnosis of chronic pelvic inflammatory disease was made and the patient treated by salpingectomy. Gross examination of the tube revealed a thickened, fibrous organ with partially obliterated fimbriate folds and whitish gray nodules in the region of the isthmus. Microscopic examination showed in the wall of the tube numerous stellate abscesses surrounded by typical masses of endothelial cells. The patient reacted positively to two Frei antigens. A very interesting case of localization of the virus in the human brain was observed by Schenken in this laboratory. A woman 45 years of age who had been suffering for some time with inflammatory stricture of the rectum was admitted to the hospital with symptoms of meningeal irritation. These were ascribed to pellagra, a frequent complication of stricture of the rectum. Autopsy and histologic examination revealed a diffuse meningeal reaction with lymphocyte and plasma cell infiltration and localized areas of superficial necrosis. Intracerebral inoculation of emulsions of this brain into white mice produced the typical picture of venereal lymphogranuloma encephalitis. The recovered virus was transmitted through several generations of white mice. Antigens prepared from these infected mouse brains gave positive cutaneous reactions in patients proved to have the disease. Lesions involving the urethra have been described in both males and females. We observed in each of 2 females a lesion of the urethra which transformed it into a rigid fibrous

tube. Biopsy showed granulation tissue with marked fibrous and lymphocytic infiltration. A Negro who came to the clinic of the State Charity Hospital of Louisiana at New Orleans because of swelling of the penis and difficulty in voiding was found to have a large granulomatous mass destroying the urethra. Microscopic examination of this mass revealed small nodules or handlike arrangements of endothelial cells interspersed with polymorphonuclear leukocytes, lymphocytes and plasma cells. Giant cells resembling Langhans cells were present. Inoculation of guinea pigs with tissue emulsions produced no evidence of tuberculosis. The patient had a positive Frei reaction. Complete autopsies on patients with venereal lymphogranuloma have been reported by: Hillsman, Wilshusen and Zimmerman; Vernich; Wien and Perlstein; Wien, Perlstein and Neiman; Lichtenstein, Reichle and Connor. Most of the reports contain nothing of interest. In the case observed by Reichle and Connor, excision of inguinal buboes histologically characteristic of venereal lymphogranuloma was followed by purulent arthritis of the hip joint. At autopsy a large psoas abscess was found with destruction of the hip joint, as well as nodular masses in the kidneys and adrenals. On microscopic examination these masses showed "mononuclear abscesses" with central necrosis caused, in the opinion of the authors, by the virus of venereal lymphogranuloma. No attempts, however, were made to demonstrate the virus by animal inoculation, nor were diagnostic antigens prepared from the tissues. We have performed autopsies in 23 cases of chronic inflammatory stricture of the rectum and have not found such lesions as described by Reichle and Connor. The only notable observation which we wish to record from our autopsy experience is the occurrence of pellagra in 22 per cent of the cases of rectal stricture. As stated in previous publications, this coincidence is, to say the least, remarkable.

Lesions in Animals.—Lesions in animals have so far been observed only after experimental inoculation of the animals with the virus, spontaneous occurrence of venereal lymphogranuloma being unknown to veterinary medicine. Owing to the recent interest aroused by this disease, there are now available many accurate accounts of such experimental lesions in various susceptible animals.

Central Nervous System.—The best method of infecting animals with the virus of venereal granuloma is by intracerebral inoculation. The principal lesion so produced is encephalomeningitis, especially pronounced in certain types of monkeys, white mice and ferrets (D'Aunoy and von Haam) but infrequent in guinea pigs and rabbits. Macroscopically one observes moderate to marked diffuse hyperemia of the brain, not restricted to the area of trauma or its immediate vicinity (Miyagawa and co-workers). Microscopically the picture is that of

dense infiltration of the meninges of the brain, occasionally those of the upper part of the cord and the brain substance itself. Neutrophilic leukocytes, large and small mononuclear cells and plasma cells arranged in cell nests around the smaller vessels give rise to the so-called lymphomas. The choroid plexus shows similar areas of infiltration, and subependymal edema is often very characteristic during the acute stage of the disease. We have frequently observed pyknotic changes in some of the ganglion cells near the areas of infiltration, but in general the damage to nerve tissue proper is slight. The histologic picture is fairly characteristic, and the lesions can be easily differentiated from those of spontaneous encephalitis, described as occurring in various animals, especially white mice. Ionesco-Mihaiesti and co-workers described, after intraperitoneal inoculations, degenerative lesions in the columns of Goll with demyelination of axis-cylinders and glial proliferation similar to the histologic changes in human tabes. We have never seen such lesions, nor had Findlay. We believe with Levaditi and his co-workers that they resulted from spontaneous disease in the animals and were not produced by the virus. Levaditi designated the experimental lesions of the brain caused by the virus as "neuromesodermoses," in contrast to the neuroectodermoses produced by neurotropic viruses. Findlay produced lesions in the brain by intraperitoneal injection of the virus and simultaneous traumatization of the brain.

Skin and Lymph Glands.—The second most commonly used method of infecting experimental animals is the intracutaneous or subcutaneous inoculation of material containing the virus. This method is especially useful with respect to monkeys, guinea pigs and rabbits. The lesion produced at the site of inoculation is quite similar to the primary lesion seen in man. Findlay described it as a thickening of the epithelial layer with dense subepithelial infiltrations of few neutrophils and many plasma and plasmacytoid cells. The endothelium of the subepithelial capillaries is swollen to such an extent that the lumens are almost closed. Levaditi and Nicolas observed similar changes. The regional inguinal glands after such inoculations show lesions comparable to the inguinal buboes of the human disease. This is especially so in the guinea pig. With monkeys, however, such infections are followed by early and extensive abscess formation (Findlay). De Blasio, by direct intraglandular inoculation of infected material, produced similar lesions in the cervical glands of guinea pigs, and Bonne and his co-workers in Netherland East Indies noted similar changes following injection of material from climatic buboes.

Changes in Other Organs.—Caminopetros and Photakis obtained marked hyperplasia of the reticuloendothelial cells in the lungs of rabbits by intrapulmonary injection of the virus. Such lesions have been simi-

larly produced in rabbits and guinea pigs (von Haam and Hartwell). Macroscopically the lungs show small grayish areas resembling atelectatic lung tissue. Microscopically there is marked accumulation of endothelial cells with round cell infiltration. A single injection will eventually produce numerous such focal lesions throughout the lung. Meyer and co-workers noted perivascular changes in the liver after intrahepatic injection of virus-containing material. Splenic enlargement usually follows injection of the virus by any route (Meyer, Rosenfeld and Anders; Nicolas; de Blasio; D'Aunoy and von Haam). Subsequent to intracerebral inoculation Levaditi, Hellerström and Wassén and Findlay were able to recover the virus from the spleen. Miyagawa and his co-workers, by intratesticular injection of virus-containing material into white mice, produced in a few cases adhesions between the tunica vaginalis and the parenchyma of the testicles with atrophic and sometimes purulent changes. Intraperitoneal inoculation causes adhesive peritonitis (Ionesco-Mihaesti and co-workers). Corneal scarification in monkeys did not produce keratitis, but injection of virus-containing material into the vitreous produced iridocyclitis with subsequent atrophy of the eyeball (Stannus). Intracerebral injection of material containing the virus into white mice, monkeys, ferrets, cats, sheep and calves produced in the majority of cases severe unilateral or bilateral conjunctivitis within from three days to two weeks (von Haam and Hartwell). Microscopically such lesions show hypersecretion of mucus by the columnar cell epithelium and infiltration by large and small mononuclear cells and sparse neutrophilic leukocytes within the subepithelial layer. Inclusion bodies have never been observed. The dissemination of the virus in animals infected experimentally is apparently dependent on the route of inoculation, intracerebral and intraperitoneal injections usually leading to general spread, while subcutaneous injection seldom produces more than a primary lesion and regional glandular swelling. The close identity of some of the lesions in animals with the lesions observed in man must be emphasized, suggesting the possibility of many yet undescribed lesions occurring in man.

ETIOLOGY

Numerous attempts have been made to cultivate the causal agent of venereal lymphogranuloma from the pus of "climatic buboes" or the buboes of "Nicolas Favre's disease." Through such efforts various types of organisms have been isolated by many investigators, although others consistently have found pus from such lesions sterile. As previously noted, most authors of the nineteenth century considered venereal lymphogranuloma as a tuberculous or syphilitic process, and some even ascribed its manifestations to *Plasmodium malariae*, *Pasteurella pestis*, fungi, diphtheroids, various types of cocci, amebas, unknown toxins,

constitutional factors and climatic influences (L. Martin; Cantlie; Ruge). The greatest difficulties under which investigators of this period worked were undoubtedly inability to select cases in which there were no complications and inability to reproduce the disease in experimental animals. These handicaps were removed by the discovery of the specific skin reaction by Frei, in 1925, and the transmission of the disease to monkeys by Hellerström and Wassén, in 1930. Shortly thereafter, these Swedish workers, as well as Levaditi, Ravaut, Lépine and Schoen, demonstrated a filtrable virus as the causal agent of the disease. In the United States Grace and Suskind and D'Aunoy, von Haam and Lichtenstein demonstrated the presence of the virus as an endemic infectious agent in New York and New Orleans, and D'Aunoy and his co-workers established the identity of their strains with the virus isolated by continental workers. Since its discovery, the presence of the virus in cases of venereal lymphogranuloma has been confirmed by authors all over the world, and the etiologic significance of the virus is now well established. According to McKinley's classification, it belongs to the group of filtrable viruses without cell inclusions.

Habitat and Routes of Infection.—The virus of venereal lymphogranuloma has been recovered from the tissues and exudates of the primary lesion (Löhe, Rosenfeld, Schlossberger and Krumeich); from the pus and tissues of inguinal buboes (Hellerström and Wassén; Levaditi and co-workers; Findlay; Cohn and Kleeberg; Freund and Reiss; de Blasio; Nicolau; Caminopetros, Phylactos and Photakis; Grace and Suskind; von Haam and Lichtenstein; Bizzozero and Midana; Miyagawa and co-workers, and others); from the tissues of the chronic lesions—esthiomène and inflammatory stricture of the rectum (Ravaut and co-workers; Laederich, Levaditi, Mamou and Beauchesne; Levaditi, Mollaret and Reinié), and from the spinal fluid of patients with the disease in acute form (D'Aunoy and von Haam). Transmission of the disease by means of the blood (Findlay; von Haam and D'Aunoy) or the saliva (D'Aunoy and von Haam) of patients afflicted with the disease has not been possible. In none of the reported cases of remote extra-genital lesions, such as cutaneous manifestations or arthritis, has the virus been proved by animal inoculation to have been directly responsible. Coutts's theory that the virus is of buccal origin is based purely on clinical evidence (Coutts; Coutts and Banderas Bianchi). A carrier state has never been demonstrated in healthy human beings.

In most cases the genital tract is the portal of entry for the virus in man. The virus is probably transmitted through direct contact with the diseased mucous membrane during coitus, numerous partner cases proving the venereal nature of the infection. Because of the evanescent character of the primary lesion in the female, this sex must be held

responsible for the greater number of cases of venereal lymphogranuloma in man. Simple contact, without sexual relations, may transmit the virus to children, as in the case reported by Luján and Rotter. Infected douche instruments have been blamed for transmission of the virus among women. The question of an intermediary animal host which may play a role in the transmission of the virus has been raised by Coutts, and he incriminated the pubic louse, met so often in patients of the low social stratum affected by venereal lymphogranuloma. So far no proof sustaining his contention has been adduced. We have unsuccessfully searched for concurrence of venereal lymphogranuloma and pediculosis pubis. Sexual perversions may produce extragenital infection, and careless handling of infectious material may bring the disease to physicians and nurses. Bonne, van der Horst and Pet reported in 1933 that diagnostic antigen prepared according to the method of Frei produced in 3 volunteers one to six weeks after inoculation typical axillary buboes, and they argued that the temperature recommended by Frei may not kill all "strains" of the virus. We have had a similar experience. A batch of heated mouse antigen produced typical axillary buboes and small pustular lesions at the site of injection. We ascribed this to carelessness in the preparation of the antigen rather than to unusual resistance to heat on the part of the virus used. Purposeful infection of human beings has been reported (Levaditi, Marie and Lépine; Levaditi, Ravaut and Cachera; Chevallier and Bernard). Only in the case reported by Levaditi and co-workers was a regional bubo produced.

The period of incubation varies between three days and three weeks. We encountered a case in which a primary penile lesion appeared two days after intercourse and another in which buboes appeared as late as four weeks after coitus. An interval of about one week usually elapses between the appearance of the primary lesion and the establishment of the inguinal bubo. One of our female patients received a slight laceration during intercourse, and an inguinal bubo developed three days later. The frequent absence of any discernible primary lesion makes accurate determination of the period of incubation extremely difficult.

PHYSICAL CHARACTERISTICS OF THE VIRUS

Filtrability and Size.—That the virus can be filtered through the usual bacterial filters has been definitely established by Hellerström and Wassén and Levaditi and his school, but filtration is not successful in every instance. This is not surprising in view of the many factors involved, such as the hydrogen ion concentration and nature of the virus emulsion, the preparation and electrical charge of the filter, the absorption of virus, etc. According to Findlay, the virus can be filtered better from emulsions of monkey brains than from emulsions of mouse brains.

We have succeeded in passing the virus in emulsions of glands from infected monkeys, mice and ferrets and from the membranes of infected eggs through Chamberlain candles L3. We have failed, however, to recover the virus from filtered pus of inguinal buboes. Miyagawa and co-workers succeeded in passing the virus with ease through such filters as Chamberlain L2 and L3, Berkefeld V and N and Seitz E. K. They also passed the virus through collodion membranes with pores smaller than 0.24 micron. Our recent experiments have shown that purification of virus suspensions by the isoelectric point method facilitates their filtration (D'Aunoy and Andes). Broom and Findlay estimated the size of the virus to be between 0.125 and 0.175 micron, similar to the size of the virus of vaccinia. Visible forms of the virus were described by Miyagawa and his co-workers. In smear preparations from human infected glands and from lesions in the central nervous systems of experimentally infected animals, they found spherical microcorpuscles, approximately 0.3 microns in diameter. They regard these not as identical with the Gamna bodies but as corpuscular forms of the virus, their minute size allowing passage through collodion, Seitz and other filters. We have not been able to confirm the observations of these Japanese authors.

Resistance to Physical and Chemical Agents.—The virus shows little resistance to heat, being inactivated when exposed to 56 C. for thirty minutes, according to Levaditi, Marie and Lépine, or for ten minutes, according to Miyagawa and co-workers. The same authors observed that it remained virulent for twenty-three days at temperatures of 4 C. and for thirty days in a frozen state. Hellerström and Wassén obtained practically similar results. According to Findlay, freezing and drying in vacuo preserve material containing the virus without loss of virulence for three months. We have desiccated infected ferret brains in vacuo at freezing temperature, stored them in the ice box (4 C.) for twenty-five days and found them still infectious for white mice. Solution of formaldehyde U. S. P. in the concentration of 1:1,000 renders the virus inactive, while phenol and sodium ricinoleate in the same concentration have only an attenuating effect on it, mice inoculated with virus suspensions so treated dying sixty to ninety days after inoculation. In contrast to the neurotropic viruses, the virus of venereal lymphogranuloma is quickly inactivated by glycerin (Levaditi and co-workers). In the presence of oxygen and light methylthionine chloride (methylene blue) and acriflavine in concentrations of 1:100,000 inactivate emulsions of virus-containing mouse brain; in the absence of light these dyes in similar concentrations have no such effect. The action of ultraviolet rays and radium has been studied by Levaditi and by Levaditi and Reinié, who found that exposure of emulsions of virus-containing matter

to the rays of a mercury vapor lamp for thirty minutes rendered them noninfectious, while exposure to the gamma rays of 7.5 millicuries of radium did not affect their virulence.

BIOLOGIC CHARACTERISTICS OF THE VIRUS

Natural and Acquired Immunity.—The problems of immunity are practically open chapters in the knowledge of venereal lymphogranuloma. Whether natural immunity to the disease occurs in man is still undecided. The marked prevalence of the disease among Negroes we attribute, in accord with other authors (C. F. Martin and others), more to the unhygienic and unsocial methods of living among them than to greater racial susceptibility. The question of permanent immunity produced by the disease or by subclinical infections with the virus is still undecided. We are of the opinion that cases of reinfection (Kitchevatz) can be explained as simple recurrences resulting from residual infection.

Antibody Production.—Without doubt, the appearance of specific sensitivity to Frei's antigen indicates the production of specific antibodies in human and animal hosts. The nature of these antibodies and their mode of action, however, have not been determined. Patients in the last stage of inflammatory stricture of the rectum react to the specific antigen in exactly the same manner as do patients with a slight or severe attack of inguinal bubo. This allergic condition persists for many years after the disease has apparently been cured and is only slightly influenced by such circumstances as old age, stage of nutrition, concurrent disease or reappearance of the disease (Nicolas, Lebeuf and Charpy). The presence of virucidal substances in the serum of patients recovering from venereal lymphogranuloma is reported by Levaditi, Ravaut, Lépine and Cachera and confirmed by Findlay. Working with guinea pigs and monkeys, Miyagawa and co-workers were also able to demonstrate virucidal substances in human convalescent serum. In our experiments serum from patients convalescing from acute inguinal buboes two to four months after infection did not neutralize emulsions of virulent mouse brains. Kalz and Sagher noticed milder evolution of the disease in patients administered serum from those convalescing from the disease. Gottlieb reported that Frei antigens are neutralized when mixed with convalescent serum and kept at ice box temperature for forty-eight hours. Gottlieb's results have not been confirmed by Miyagawa and co-workers, Gallego Calatayud or ourselves.

Plurality of Virus Strains.—The question of a plurality of virus strains in this disease is as yet undecided. Coutts concluded from his clinical studies that there are two different types of the virus, each producing various syndromes of the disease. Virus A, according to Coutts, is the etiologic factor of a strictly localized disease, "lympho-

granulomatosis venerea," while virus B produces a systemic infection of the lymphatic system, "lymphopathia lymphogranulomatosa." We have not been able in our extensive studies to find differences in the virus as isolated from various sources which would warrant such a belief. We admit, however, in agreement with Chevallier and Bernard, that we have found differences in aggressiveness and in invasiveness in the virus as variously isolated.

Animal Susceptibility.—After numerous failures and doubtful results Hellerström and Wassén reported at the session of the Eighth International Congress of Dermatology, in 1930, that they had succeeded in transmitting the disease to *Macacus rhesus* and *Macacus cynomolgus* by intracerebral injections of pus obtained from buboes of patients. Since this fundamental work numerous reports recording susceptibility of various animals to the virus have appeared. The following species of animals have been studied in regard to susceptibility: monkeys (de Bellard; Hellerström and Wassén; Levaditi and co-workers; Cohn and Kleeberg; Löhe, Rosenfeld, Schlossberger and Krumeich; Caminopetros, Phylactos and Photakis; Ionesco-Mihaiesti and co-workers; Findlay; D'Aunoy, von Haam and Lichtenstein; Grace and Suskind; Miyagawa and co-workers); rabbits (Chevallier, Lévy-Bruhl and Moricard; Freund and Reiss; Levaditi and co-workers; Caminopetros, Phylactos and Photakis; Findlay); guinea pigs (Ravaut, Boulin and Rabeau; Gay Prieto; de Blasio; Meyer, Rosenfeld and Anders; Freund and Reiss; Nicolau; Caminopetros, Phylactos and Photakis; Findlay; D'Aunoy, von Haam and Lichtenstein; Bizzozero and Midana); dogs (Nicolau; Findlay); cats (Levaditi, Ravaut, Schoen and Vaisman; von Haam and Hartwell); white rats (Findlay); field voles (Findlay); ground squirrels (Caminopetros and co-workers); ferrets, sheep, calves and frogs (D'Aunoy and von Haam). Of these, white mice, certain species of monkeys (*M. cynomolgus*, *Hapale penicillata* and the baboon) and ferrets are highly susceptible to the virus, a large percentage of these animals succumbing to its effects. Only a small percentage of dogs, cats and guinea pigs can be infected, while inoculation of rabbits, sheep and calves produces infection in rare instances. Chickens, ground squirrels and field voles may harbor the virus for ten to thirty days without presenting any symptoms or lesions, and in frogs and white rats the virus is quickly destroyed and does not affect the animals.

Passage of the virus from animal to animal is possible, the "Kamm" strain having been preserved in Levaditi's laboratory for over five years. In our laboratory, we have kept six strains highly virulent by biweekly transfer to new animals for four years. Repeated passage through the same species of animal may lead to increased invasiveness (Grace and Suskind) or in rare instances to autosterilization of the virus strain

(Levaditi). Passage from one species to another has been performed without difficulty, and passage from animal back to man has been successful (Levaditi, Ravaut, Lépine and Schoen). The type of lesion produced experimentally in the susceptible animal depends on the portal of entry of the virus. The most effective route of infection is the intracerebral one, wherein it is of little importance whether the inoculum be deposited subdurally or intracerebrally. The essential pathologic condition produced thereby is meningoencephalitis, which proves fatal to the majority of animals. General spread of the virus throughout the body following intracerebral inoculation has been proved to occur by Levaditi and Reinié. It was recovered from other organs after intracerebral infection of the animals by Levaditi, Ravaut, Lépine and Schoen and by D'Aunoy and von Haam. Meningoencephalitis can also be produced by intraperitoneal inoculation of the virus if the brain is simultaneously traumatized by injection of a sterile starch emulsion (Findlay). Bilateral fibrinopurulent conjunctivitis has been encountered with such regularity in intracerebrally infected animals that it must be regarded as significant (von Haam and Hartwell). Subcutaneous inoculation of the virus in monkeys, dogs, rabbits and especially guinea pigs produces at the site of injection a small papular lesion, with swelling of the regional glands, in about 30 per cent of cases. Occasionally generalized infection may follow such an injection (Meyer, Rosenfeld and Anders; Nicolau). Intraperitoneal inoculation (Mihaiesti and co-workers) may produce severe adhesive peritonitis. Intrapulmonary inoculation, according to Caminopetros and Photakis, produces characteristic lesions of the lungs in rabbits. Intravenous, intraocular, intraarticular and intranasal inoculations fail to establish the disease (Levaditi and Reinié). From emulsions of the organs of infected and diseased animals, potent antigens which can be used successfully for the Frei diagnostic reaction in human beings can be prepared (D'Aunoy, von Haam and Lichtenstein).

Cultivation of the Virus.—Successful cultivation of the virus in vitro was reported by Tamura, who claimed to have grown it by Maitland's method. The fluid portion of his cultures when heated served, he found, as excellent antigens for Frei's specific diagnostic reaction. Applying Tamura's method, we placed 0.1 cc. amounts of 20 per cent emulsions of infected mouse brains in Maitland tubes. Two to three days after inoculation a distinct cloudiness appeared in the tubes. From the second and third subcultures mice were inoculated intracerebrally with 0.1 cc. amounts of the supernatant fluid. In these mice characteristic histologic changes developed. We do not believe that this evidences virus growth. The dilution affected by subculturing was still well within the limits within which the material could prove infectious. Recent reports by Voet and by Miyagawa and co-workers fail to confirm Tamura's results.

Cultivation of the virus in the chorioallantoic membranes of hatching chick embryos was reported by Miyagawa and associates. These workers, employing the technic described by Goodpasture, Woodruff and Buddingh, were able to propagate the virus for five generations. Smears from the white plaques observed on the membranes after inoculation contained granulocorpuscles similar to those previously described by Miyagawa in human material. We have carried on similar experiments during the past two years and have been able to propagate the virus on the chorioallantoic membrane. We were unable to find specific histologic changes that could be ascribed to virus action, and interpreted the "bodies" described by Miyagawa as products of cell degeneration resulting from nutritional changes occurring during the normal development of the chorioallantoic membrane, which, it should be remembered, is an organ of excretion and respiration in the developing chick (D'Aunoy and Evans).

DIAGNOSIS

In general it may be said that the diagnosis of a disease can be made by four different methods: clinical methods, by which the manifestations and symptoms are observed and interpreted; bacteriologic methods, by which the causal agent is demonstrated; pathologicoanatomic or histologic methods, by which the tissue changes are evaluated and interpreted, and immunologic methods, by which the host's reaction to the noxious agent is elicited.

The diagnosis of venereal lymphogranuloma by purely clinical methods is possible in many cases but in our opinion is always difficult. In most cases a definite diagnosis cannot be reached by such methods alone. We have already discussed the principles underlying the clinical, bacteriologic and histologic diagnosis in the chapters dealing with the manifestations of the disease, the causal agent and the pathologic picture. In this chapter, therefore, we shall refer only to the immunologic diagnosis of venereal lymphogranuloma, discussing especially the technic and the diagnostic value of the Frei intracutaneous reaction.

Technic of the Frei Test.—Frei recommended, as proper material for the preparation of diagnostic antigen, bacterially sterile pus aspirated from the bubo of a patient suffering from venereal lymphogranuloma. This pus he mixed with four to six parts of physiologic solution of sodium chloride and inactivated the emulsion at 60 C. for two hours one day and for one hour the next day. Only after bacteriologic tests showed the material to be sterile did he consider it fit for use. Dind and Hellerström and Wassén made use of extracts of glands extirpated from patients with venereal lymphogranuloma under aseptic precautions. With these they obtained results that were as good as those obtained with emulsions of pus. In 1931, after Hellerström and Wassén had

isolated the virus of venereal lymphogranuloma, the former prepared emulsions of brain substance from monkeys suffering from meningo-encephalitis following intracerebral injection of gland material from a patient with venereal lymphogranuloma. Such emulsions gave positive intracutaneous reactions in patients who had venereal lymphogranuloma and negative results in controls. Later Findlay and Wassén independently recommended the white mouse as the most practical animal for the preparation of antigen. Bloom, Kleeberg, and Grace and Suskind also recommended such antigenic material, the latter emphasizing that brain emulsions desiccated in vacuo at freezing temperature constituted by far the best material for use in the diagnostic skin test.

Although both types of human antigen proved generally satisfactory, we found difficulty in standardizing various batches of antigen prepared from material secured from different patients. Pus obtained from some patients possessed much weaker antigenic properties than that from others. We therefore adopted emulsions of brains from animals experimentally infected with the disease as standard antigen, and the use of this antigen prepared as a routine in our laboratories has proved the method of choice in obtaining acceptable results over a long period. While numerous animals are susceptible to the disease, only infected monkeys (common marmoset or *Hepale penicillata*) and white mice have proved useful as sources of brains for antigenic brain emulsions.

Since our clinical experiences suggested the possibility of virus strains of various aggressiveness (Chevallier and Bernard), we chose from 40 strains carried in our laboratories for from four to twenty-six animal passages the 6 which produced the strongest reactions in animals for use in the production of antigen. These 6 strains have been carried through mice by biweekly intracerebral injections and have remained unchanged in power of invasiveness for these animals. The inoculated dose is such that at the end of two weeks the infected animals show signs of sickness with marked histologic changes in the brain and meninges, but only few die.

The preparation of antigen from the brains of these animals follows the general outline published by Frei and by Hellerström:

A 10 per cent emulsion of brain in saline solution is heated to 52-54 C. for two hours the first day and for one hour the second day. Five tenths per cent phenol is used as a preservative, and tests for sterility are performed repeatedly in order to avoid using infected material. Antigens prepared from the various strains are pooled, the stock mixture containing all 6 strains. Stock antigen is kept in the ice box (4 C.) in the dark and the "date of expiration" set empirically at six months. Samples of older batches are always available for control tests. Before being used for diagnostic purposes, each batch of mixed stock antigen is tested for specificity and sensitivity. At least six tests on persons known to have venereal lymphogranuloma and six control tests on patients not infected are performed

and the results compared with those obtained with other stock antigen mixtures and human antigens.

By these means we have succeeded in obtaining antigen of high specificity and sensitivity which always produces the same type of reaction when tested on the same patients under similar conditions. It is our belief that the inactivating temperature of 60 C. recommended by Frei in the preparation of the antigen is too high and does not result in production of the best diagnostic antigen, judged by the criteria of specificity and sensitivity.

The technic which we employ for the Frei test follows in general the directions given by Frei:

After the forearm has been cleansed with alcohol, 0.1 cc. of the antigen is injected intradermally through a fine needle. This produces a small weal from 3 to 5 mm. in diameter. On the same arm the same quantity of an emulsion of normal brain from healthy white mice, which has been prepared and treated in the same manner and is of the same age as the antigenic emulsion, is similarly injected as a control. (The use of an emulsion of healthy mouse brain as the control for the antigen prepared from infected mouse brain is absolutely necessary and preferable to the injection of any other control material.) The reaction is read after forty-eight hours and reported as doubtful, positive, strongly positive or negative. A doubtful reaction is not regarded as diagnostic, and the test is repeated. If possible, a second reading is made on all reactions after four days. The site of the control injection at the time of the reading of the reaction usually shows only the mark of the needle with sometimes a small deposit of unabsorbed material. The positive reaction consists of an elevated and inflamed weal measuring 0.7 to 1 cm. in diameter. The strongly positive reaction shows a red edematous area measuring several centimeters in diameter and usually containing centrally placed small yellowish areas of necrosis. The positive reaction is discernible for several days and in some instances heals with distinct pigmentation at the area of injection.

Histologic examination of tissue obtained from the site of a positive Frei reaction shows marked hyperemia with vascular dilatation and perivascular accumulation of lymphocytes and plasma cells but comparatively few neutrophils. The epithelium shows some edema but otherwise not much damage. Only in very severe reactions is there necrosis of the epithelium and of the subepithelial structures. We have not been able to demonstrate inclusion bodies or bacteria in these lesions. Sézary, Lévy-Coblentz, Mauric and Lenègre described an identical picture and emphasized the close similarity to the histologic picture of the Dmelcos reaction for infection with Ducrey's bacillus.

The Diagnostic Value of the Frei Reaction.—The introduction of the specific skin test by Frei in 1925 can rightly be hailed as a "milestone of great importance" (Stannus) in the diagnosis of venereal lymphogranuloma. It stimulated tremendously further studies of the disease. With the aid of this test, the etiologic relationship between venereal

lymphogranuloma and climatic bubo (Findlay), inflammatory stricture of the rectum (Frei and Koppel) and nonspecific urethritis of the Waelsch type (Frei, Wiese and Klestadt) was demonstrated and the complete clinical entity of this virus disease ultimately established. Since the condition of allergy which is the cause of the positive reaction of the skin remains for many years after healing of the lesions—perhaps even throughout the life of the patient—a positive reaction does not indicate the presence of active disease and has perhaps less differential diagnostic value than a negative reaction, which definitely excludes the infection, active or latent. This fact must be borne in mind, especially if a positive reaction is obtained in an older person. In such a case the possibility of a previous infection with venereal lymphogranuloma should always be excluded by careful consideration of the past history. On the other hand, some time elapses after infection before the specific allergic state of the host is reached; therefore the false negative reaction is encountered frequently, particularly in patients with the infection in a very acute stage and in those in whom allergic responses are slow. These factors explain the majority of discrepancies which have been reported between the clinical findings and the results of the Frei test (Gaté and co-workers). Careful evaluation of the clinical history and repetition of the test after a lapse of some time will clear most such apparent discrepancies and considerably increase the diagnostic value of the Frei test.

Many investigators (Frei and his school; Hellerström; Hermans; Jersild; Löhe and Blümmers, and others) have confirmed the specificity of this test in numerous cases of the disease. Frei, Wiese and Klestadt emphasized the importance of the proper selection of the case in which to obtain the material from which the antigen is prepared, and his careful criticism of all reports in which doubt had been expressed as to the specificity of the reaction bearing his name brings out several important points, such as coexistent venereal infections and faulty preparation of the antigen.

The antigen must be prepared from material obtained from a patient with the disease in a typical form, and its bacterial sterility should be tested repeatedly. Control tests should always be made in order to exclude false positive reactions resulting from general skin hypersensitivity. The material used for control tests must be homologous to the specific antigen. If emulsions of brains of infected animals are used, it is essential that emulsions of normal homologous brains be used for control injection in order to avoid false positive reactions due to the sensitivity of the skin to the brain substance of the animal (von Haam and Hartwell).

Our results with the Frei test have been most satisfactory. Tests performed on over 500 patients with clinical venereal lymphogranuloma

and over 800 controls who did not have this disease gave 98.1 per cent correct results. Of the positive reactions, only 7.2 per cent were "weakly positive" (D'Aunoy and von Haam). After distributing portions of our standard mouse antigen to interested physicians in various parts of the country, we obtained from 90 per cent of these physicians reports of results in complete agreement with clinical findings. These results are in accord with the observations of Flandin, Rabeau and Turiaf, who reported 1,170 Frei reactions on 400 patients with 95 per cent correct results. Smaller series published by others (Dalton; Curth; Cole; Grace and Suskind; Vander Veer and co-workers) gave 100 per cent correct results. An extensive review of the evidence for the diagnostic value of the Frei test, presenting statistics from 41 other clinics, was reported to the American Proctologic Society by Bacon, of Philadelphia. Only 6 of the various contributing authors reported satisfactory results in less than 90 per cent of their cases, the most unsatisfactory results being in cases of rectal stricture. Repeated Frei tests in patients with venereal lymphogranuloma observed for two years demonstrate, according to Bacon, that one negative test should not be considered as sufficient evidence to exclude venereal lymphogranuloma if the clinical evidence speaks for its presence.

Simon, Braley and Minck applied the Frei test to 50 female patients picked at random and obtained 12 per cent positive results; only a single patient showed an esthiomène-like lesion on the vulva which could be considered clinically as evidence of infection with the virus. Their conclusion that the Frei reaction gives erroneous results in 10 per cent of patients tested is sharply criticized by Sézary and Lenègre, who point to the possibility of latent or veiled infections, especially in females. Weiss and Kuntzmann reported a series of cases in which histologic observations bore out all positive Frei reactions. We have had similar experience in all cases in which patients with positive Frei reactions were operated on. Levaditi, Durel and Reinié observed differences in the strength of antigens prepared from emulsions of brains of different monkeys. We have made similar observations. In an interesting report Strauss and Howard claimed that antigens prepared from brains of infected mice give false positive reactions some time after their preparations, as will antigens prepared from brains of normal mice. On the basis of 217 Frei tests made on persons suffering from acute venereal lymphogranuloma and on healthy medical students in which mouse brain antigens of varying age were used, von Haam and Hartwell concluded that neither the specificity nor the sensitivity of such antigens is altered by storage in the ice box (4 C.) or at room temperature (20 C.) for as long as fourteen months.

Sullivan and Ecker criticized the dose of antigen injected, warning that heavy doses may easily produce nonspecific reactions. In reply

Hellerström and Wassén stressed the fact that specific reactions appear delayed; i. e., they appear after forty-eight hours and remain evident for as long as eight to twelve days in contradistinction to nonspecific reactions, which appear earlier and are evanescent.

Coutts recommended as a valuable control for the Frei test a specific complement fixation test for venereal lymphogranuloma; human pus or lymph gland emulsion is used as antigen (Coutts, Landa Perroni and Martini Herrera). On the basis of 300 parallel tests Coutts and Ponce held that any positive Frei reaction appearing about twenty-five days after the onset of the infection is specific. Prats was not able to confirm this conclusion. Gottlieb's report of antigen-neutralizing bodies in the serum of convalescent patients has not been confirmed (Miyagawa and co-workers; Sullivan and Ecker), nor has the observation of Reiss concerning the antigenic properties of human serum obtained in the second or third week of the disease.

THERAPY

There is no better means of expressing the present knowledge of the therapy of venereal lymphogranuloma than to quote the words with which the great continental expert on this disease, Sven Hellerström, introduced his answer to a questionnaire on the subject for a dermatologic journal: "It is a problem which at present is far from being solved even in the hands of the trained specialists." Nevertheless, current medical literature contains an amazing amount of material recommending treatment of the disease by widely different methods. To the critical reader of these reports, however, three facts are evident: 1. Many authors are unaware of the true character of the disease. 2. Comparative investigation of various therapeutic methods by the same author is rare. 3. In most instances the number of patients reported treated is small.

On the basis of clinical and experimental observations we rightly assume that a passing general infection of the organism with the virus is frequent and leads to constitutional symptoms and under certain conditions produces distant lesions in the joints, skin, eyes and other organs (Coutts; von Haam and D'Aunoy). In a certain number of cases, months or years after the acute symptoms of the disease have subsided there occurs elephantiasic enlargement of parts of the external genitalia (labia or scrotum), sometimes with extensive ulceration, followed frequently, especially in the female, by inflammatory stricture of the rectum and severe proctitis. This is still thought by the majority of authors to be a consequence of the destruction of the lymph drainage (Stannus); some, however, regard it as a manifestation of the action of active virus, which still can be demonstrated in the diseased tissues by inoculation of animals (Coutts). While the disease rarely leads to the

death of the afflicted person, it brings much discomfort. Many of its victims are for months or years unable to do any work; they fill the charity clinics and the welfare institutions. Since methods of preventive medicine and public hygiene are still powerless to prevent this infection, it appears logical that every effort should be exerted in attempting to combat the disease successfully when once it is established.

The therapeutic methods advocated can be divided into three general groups: physical, medical and surgical. Gay Prieto emphasized that no treatment is equally successful for all the manifestations or types of venereal lymphogranuloma, and Rousseau and Adamesteanu warned against adopting any one form of therapy. It is advantageous to discuss separately the therapy recommended for the early and that for the late or secondary manifestations of venereal lymphogranuloma.

Treatment of Acute Venereal Lymphogranuloma.—The primary manifestations of venereal lymphogranuloma—small herpetiform lesions, mild urethritis or, in rare instances, phagedenic ulcers—show a marked tendency to heal spontaneously and do not require special therapeutic measures. In most cases the lesion is so minute that it is completely overlooked by the patient, and no evidence of it can be found at the time of examination. The severe constitutional symptoms which are usually present at the onset of the disease can be effectively combated by such general therapeutic measures as rest in bed, adherence to a light diet and purgation. In many cases we observed a favorable action of salicylate, especially acetylsalicylic acid. Quinine, which had been used extensively before the true character of the disease was known (Lesueur-Florent), has no place in the therapy of venereal lymphogranuloma. Most therapeutic efforts have been centered on the specific regional lymphadenitis, which is the principal manifestation of acute venereal lymphogranuloma. The evaluation of methods used in the treatment of lymphogranulomatous adenitis is, as Stannus correctly emphasized, greatly handicapped by the fact that abortive forms occur with much more frequency than has been previously assumed. This may also be the reason why early treatment of the climatic bubo, which includes all the abortive forms, is more successful (Hellerström) than treatment after fluctuation has been observed.

The physical methods employed in the treatment of the acute bubo of venereal lymphogranuloma comprise chiefly galvanic cauterization (von Veress) and irradiation of the bubo with ultraviolet rays, roentgen rays (Nicolas and Favre; Löhe and Blümmers) or radium. None of these methods is credited with outstanding success and should be applied only in combination with medical or surgical treatment.

The medical therapy consists of the use of drugs or biologic products, such as specific and nonspecific vaccines and proteins. Among the

drugs, the antimony preparations have been used most widely in the treatment of acute buboes and have, according to some investigators, proved rather successful. Antimony and potassium tartrate and various commercial preparations (stibenyl [sodium acetylparaaminophenylstibinate], neostibosan [the paraaminophenylstibinate of diethylamine], anthiomaline [the lithium salt of stibiothiomalic acid] and fuadin [sodium antimony biscatecholdisulfonate]) are injected intramuscularly or intravenously, and healing, sometimes without suppuration, is reported to occur in from four to sixteen weeks following their use (Sézary and Lenègre; Sézary, Bolgert and Joseph; Nicolas, Favre, Pétouraud and Chaniel). A strong drug reaction is desirable (Sorley and Gibson), and nausea, muscular pains and occasionally severe albuminuria have been produced by rapid injection of such drugs (Mamou). Sézary and Lenègre, on the basis of their experience, doubted the therapeutic value of the antimony preparations. Our own experience with antimony and potassium tartrate confirms the opinion of the French authors to such an extent that we have come to use failure of this type of therapy as a means of differentiating the chronic lesions of venereal lymphogranuloma from similar lesions caused by granuloma inguinale, for which antimony and potassium tartrate is specific. The numerous other remedies recommended by various authors have not been investigated sufficiently to establish their therapeutic value. Iodides (Giacardy), methylthionine chloride (methylene blue) (Araujo), various copper salts (Frei and Wiese), introcid (a cerium-iodine) (Grippain), sodium salicylate (Chevallier and Fiehrer; Touraine and Aubrun), gold salts in the form of solganol (the disodium salt of 4-sulfomethylamino-2-auromercaptobenzene-1-sulfonic acid) (Löhe and Rosenfeld) and glycerin (Pinard and André) have been tried in a small number of cases with varying results. As the most harmless of the drugs mentioned, sodium salicylate has recently found wide use, especially by French dermatologists. It is recommended (Chevallier and Fiehrer) in oral doses of 6 to 8 Gm. a day, accompanied by a strict milk diet in order to avoid gastric complications, or in the form of intravenous injections of sodium salicylic gluconate. Acetylsalicylic acid given in small doses has with us proved an excellent agent in combating the severe constitutional symptoms usually present at the onset of the disease.

With increasing frequency, attempts to treat venereal lymphogranuloma with specific or nonspecific vaccines or proteins are reported. The fact that these agents are usually less harmful than the heavy metals recommends at least their trial, although the number of reported "cures" is at present not sufficient to warrant any conclusions. Injections of milk (Tissot), T. A. B. (typhoid, paratyphoid A and paratyphoid B) vaccine (Hanschell, Lorn and Cooke), tuberculin and pyripher (a non-specific protein mixture prepared with extracts of fever-producing

bacteria from nonpathologic stocks) (Löhe) apparently have a tendency to hasten the maturation of the glands, while specific vaccines prepared from pus or emulsions of glands from persons afflicted with the disease or from organs of animals experimentally inoculated with the virus are supposed to bring about resolution of the process without supuration. Hellerström, who initiated specific vaccine therapy for venereal lymphogranuloma, recommended slow desensitization of the allergic patient by small repeated intravenous injections of Frei's antigen (Chevallier and Fiehrer; Ravaut, Levaditi and Maisler), and Gay Prieto, as a result of his experience in 3 cases, looked on this treatment as the method of choice. Ionesco-Mihaiesi, Longhin and Wisner observed healing of the lesions in 6 cases after one to five intravenous injections of from 0.2 to 0.5 cc. of antigen. Wien and Perlstein reported marked improvement of patients even after a single diagnostic intradermal injection. Kalz and Sagher reported that in 30 patients treated by intramuscular injections of convalescent serum healing of the lesions occurred in about six weeks, and Tamura recommended treatment of acute buboes with specific antilymphogranuloma goat serum—a method which resulted in cure in 3 cases after an average duration of the disease of eight weeks.

Surgical treatment of the infection in the acute stage must still be regarded as the most popular method of therapy, although it has recently been criticized severely by those who regard venereal lymphogranuloma as a systemic infection. Simple aspiration of the fluctuating glandular masses with or without subsequent injection of an antiseptic solution, broad incisions with drainage, and more or less extensive extirpation of the enlarged glands were used long before the causal agent of the disease was known. That surgical therapy still holds its place in the treatment of acute venereal lymphogranuloma can be seen from the fact that in many of the recent publications advocating different methods of treatment reference is still made to surgical treatment as a last resource in dealing with obstinate lesions. Opponents of surgical intervention (Löhe; Sézary and Lenègre) emphasized such undesirable end results as slow-healing wounds and, as a consequence of the destruction of the lymph drainage, postoperative elephantiasis of the genitalia. Those in favor of surgical treatment (Rousseau and Adamesteanu; Ruge) stressed the great acceleration of complete healing (three to six weeks) with immediate relief of local pain as great advantages. While we with others (Coutts; Stannus) have shown that general infection occurs in the course of venereal lymphogranuloma in a rather large number of cases, we have noted also that only local lesions can be observed in the majority of patients, owing to the rapid destruction of the virus in the human organism. Removal of the local lesions, therefore,

would remove the main source of possible further spread of the infection and is a biologically correct method of treatment. This fact will gain in importance if it is ever proved that the virus of venereal lymphogranuloma, like other true pathogenic viruses, multiplies only intracellularly. Another possible advantage of surgical therapy was pointed out by Jersild, who observed that partial adenectomy or only simple incision of a superficial bubo results in marked improvement in the deeper seated lymph glands. He explained this observation, which was confirmed by Hellerström, Rousseau and others, on the basis of auto-vaccination with antigen through the operative wound. The necessity of surgical intervention in cases of secondary infection with massive suppuration is generally admitted.

In our investigation of appropriate methods of treatment, 85 patients in whom the disease had been diagnosed clinically and immunologically were submitted to operative procedures and carefully observed as regards healing of the lesions and general improvement. In order to avoid the abortive form, which will heal under any therapy, we did not follow Hellerström's advice of early operation but treated all patients conservatively until the size and the consistency of the glands made spontaneous regression of the lesions appear improbable.

Partial adenectomy resulted in healing of the lesions one month after operation in over 50 per cent of the cases. Simple incision with drainage delayed the healing process in the majority of cases for from two to four weeks. Nineteen cases of abortive venereal lymphogranuloma came under observation during the period of study. In these the infection was extremely mild, without suppuration. Disappearance of the glands, or spontaneous cure, was observed in the majority of these cases after four to six weeks—a period as long as was required for healing of the acute suppurating bubo after surgical therapy. The average period of the disease was four and seven-tenths weeks for the cases in which treatment was by partial adenectomy, five weeks for those in which treatment was by incision and drainage and six and two-tenths weeks for those in which the disease was abortive, with spontaneous cure. Eight months after conclusion of the investigation none of the 85 surgically treated patients had chronic edema or elephantiasis of the genitalia, although in 27 per cent bilateral lesions were still present. These results led us to conclude that surgical intervention in acute venereal lymphogranuloma can be regarded as a successful method of therapy, partial adenectomy resulting in earlier healing of the suppurating bubo than simple incision and drainage.

Treatment of Chronic Venereal Lymphogranuloma.—While therapeutic management of the acute lesions of venereal lymphogranuloma can claim at least some degree of success, treatment of the late or chronic

lesions gives most unsatisfactory results. Modern concepts of the evolution of this infectious venereal disease, however, may explain the failure in the therapy of its chronic manifestations—*esthiomène*, or ulcerative elephantiasis, and inflammatory stricture of the rectum. Except in the rare cases in which general dissemination of the virus can be established, the infectious agent inhabits chiefly the lymph glands in the region of the primary infection and has a tendency, after destruction of the glandular tissue, to spread locally. Through lymph stasis in the tissues in which the lymph drainage has been interrupted by destruction of the glands, chronic edema is produced. The inflammatory process spreading retrograde along the lymph vessels and in adjacent tissues results in the production of a characteristic granulation tissue with numerous small abscesses and sinuses, the lesion showing a marked tendency toward massive hyperplastic fibrosis. The development of chronic manifestations depends largely on the location of the affected lymph glands, which correctly must be regarded as the center of the pathologic process. If the inguinal, presymphysial or crural lymph nodes are involved, the external genitalia (scrotum, penis and vulva) bear the brunt of the spreading infection, and elephantiasis of the affected part will develop. Infection of the anorectal, deep iliac and sacral glands causes disease of the perineum and rectum—the genitoanorectal syndrome, or venereal lymphogranuloma of the perineum and inflammatory stricture of the rectum. The skin and mucous membranes covering the edematous tissues suffer considerably from nutritive disturbances caused by the chronic lymphedema, and this allows penetration of secondary invaders into the subepithelial structures. Thus severe infections are established in already altered tissues, hastening their complete destruction. This secondary infection of tissues infected with the virus of venereal lymphogranuloma is an important complication, producing severe ulceration in the instances of elephantiasis—described as *esthiomène*—and severe proctitis in the cases of rectal stricture.

From this brief sketch of the development of the chronic lesions of venereal lymphogranuloma it is possible to realize how futile therapy will be when the process is advanced, the lesions then often representing merely an end result of the disease complicated by various secondary pyogenic infections. The only relief the patient can expect is a temporary cure of the ulcers in the rectum and on the genitalia. The truth of this statement is well borne out by practical experience in the treatment of *esthiomène* and rectal stricture. Surgical excision of the parts suffering from elephantiasis is the ideal way of treating *esthiomène*, but it is only possible when the areas of the genitalia involved are small. Antisyphilitic therapy and the injection of antimony and potassium tartrate have met more with failure than with success and cannot be

recommended. The specific treatment with vaccines or Frei antigen has not been studied sufficiently to permit any conclusion, but in the few cases in which it has been used it has not given very encouraging results. There is no effective method of treatment for rectal stricture. According to C. F. Martin, the disease in this stage may be described as incurable, tending inevitably toward a fatal termination. Resection of the rectum, sigmoid, perirectal tissues and perineum has brought permanent relief but is a drastic procedure (Lee and Vander Veer). Simple extirpation of the rectum does not remove the principal site of the pathologic process, and therefore recurrence will be observed in a large number of the cases in which this treatment is tried (Gatellier and Weiss). Colostomy is often a necessary emergency procedure and in most cases must remain permanent. It carries a mortality of about 30 per cent. Gohrbrandt suggested sloganol (the disodium salt of 4-sulfomethylamino-2-auromercaptobenzene-1-sulfonic acid) for the treatment of rectal stricture in the early stage, and G. M. Brown observed favorable results from the use of diathermy. Gay Prieto observed a case in which distinct improvement followed intravenous injection of Frei antigen. A similar observation was made by Alley, who treated 9 patients for mild stricture of the rectum with intradermal injections of antigen and obtained "encouraging results." In the Charity Hospital of Louisiana at New Orleans repeated dilatation of the stricture is the adopted procedure in all cases in which the condition is not too far advanced, and it has met with fair success in that it delays the more serious symptoms, which as a rule require colostomy.

Attention should be directed most intensively toward prevention of this incurable form of venereal lymphogranuloma through proper therapeutic management of the infection in the acute stage. In the female this stage is frequently not discernible, owing to the deep location of the affected glands, which also prevents possibly effective early treatment, and this explains the greater prevalence of chronic, inoperable, incurable venereal lymphogranuloma in such patients.

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Notes and News

University News, Promotions, Resignations, Appointments, Deaths, Etc.—Columbia University has conferred the title of professor emeritus on Francis Carter Wood, director of the Crocker Institute of Cancer Research.

Lieut. Col. A. Parker Hitchens, Medical Corps, U. S. A., has been appointed George S. Pepper professor of public health and preventive medicine in the University of Pennsylvania.

Alexander S. Wiener, Brooklyn, has been appointed bacteriologist and serologist in the office of the chief medical examiner of New York.

Alexis Carrel, Karl Landsteiner, Phoebus A. T. Levene, W. J. V. Osterhout and Florence R. Sabin are retiring from active work in the Rockefeller Institute for Medical Research, according to the retirement rule.

Frederick P. Gay, professor of bacteriology, Columbia University, New York, and William B. Castle, associate professor of medicine, Harvard Medical School, Boston, have been elected members of the National Academy of Sciences.

Francis Amory Prize.—In compliance with the will of the late Francis Amory, of Beverly, Mass., the American Academy of Arts and Sciences announces a septennial prize for outstanding work on the alleviation or cure of diseases affecting the human genital organs, to be known as the Francis Amory Septennial Prize. The prize may be awarded to any person or group for work of "extraordinary or exceptional merit" in this field. In case there is work of a quality to warrant it, the first award, which will exceed \$10,000, will be made in 1940. It may be given in one or more awards. Further information will be furnished by the Amory Fund Committee, care of the American Academy of Arts and Sciences, 28 Newbury Street, Boston.

Dazian Foundation.—The Dazian Foundation for Medical Research is prepared to award fellowships to persons holding the degree of Doctor of Medicine for the purpose of postgraduate study and research, and grants to laboratories, hospitals and similar institutions for research in medicine. Applications and inquiries should be directed to the Secretary, 180 East Sixty-Fourth Street, New York.

Society News.—The Chicago Pathological Society has elected the following officers: S. A. Levinson, president; George Rukstinat, vice president; Edwin F. Hirsch, secretary, and A. A. Goldsmith, treasurer.

Awards.—The George M. Kober medal was presented to George H. Whipple at the meeting of the Association of American Physicians in Atlantic City, May 3.

James B. Herrick has been awarded the distinguished service medal of the American Medical Association.

The Burdick Award for an "outstanding contribution to medical science" was given by the American Society of Clinical Pathologists at its recent meeting in St. Louis to Harry Goldblatt for his work on hypertension and the relation between high blood pressure and renal disease.

Abstracts from Current Literature

TO SAVE SPACE THE ORIGINAL TITLES OF ABSTRACTED ARTICLES SOMETIMES
ARE SHORTENED

Experimental Pathology and Pathologic Physiology

EXPERIMENTAL STREPTOCOCCIC ENDOCARDITIS. R. A. KINSELLA and R. O. MUETHIER, Arch. Int. Med. 62:247, 1938.

Seventeen dogs were subjected to operations whereby the mitral valve or the chordae tendineae were cut. All these animals were then fed with living cultures of nonhemolytic streptococci, which were either mixed with their food or given by stomach tube. Ten of the animals became sick; cultures of their blood showed growth of hemolytic streptococci, and the animals died. At autopsy these infected dogs had bacterial endocarditis. The bacteria in the vegetations were determined to be identical with those which had been fed to the animals. Streptococcic endocarditis can be produced in dogs with injured cardiac valves by feeding them streptococci. The fact is thus established that bacteria entering the animal body through the mouth may become implanted on an injured area within the body. The exact route which these bacteria follow has not been determined.

The reproduction of streptococcic endocarditis is complete. The success of two different drugs in curing the disease in dogs while failing to cure the disease in human beings does not obscure the identity of the experimental disease. On the other hand, the difference between the mode of production of the experimental disease and the mode of production of the disease in human beings is emphasized. This difference lies in the fact that bacterial implantation begins after an injury produced by trauma in the one instance and as a result of disease in the other. It seems highly important to collect a series of animals cured of streptococcic endocarditis and then to study the treatment after reinfection of the healed scars of previous infection. This will be a tedious task. The most interesting by-product of the present study has been the demonstration of infection of traumatized valves through feeding streptococci to animals. This part of the work, repeated in different years, seems adequately authenticated. It is of further interest that none of the microscopic appearances in the heart or elsewhere was such as to suggest a relation between the lesions observed in dogs and those of rheumatic fever which appear in human tissues.

FROM AUTHORS' SUMMARY.

EXPERIMENTAL STAPHYLOCOCCUS OSTEOMYELITIS. R. H. S. THOMPSON and R. J. DUBOS, J. Exper. Med. 68:191, 1938.

The results indicate that it is possible to produce consistently inflammation of the bones of rabbits merely by injecting intravenously a suitable strain of Staphylococcus, without resorting to any elaborate operative technic designed to localize the organisms in the bones. It appears also that the inflammatory process so produced bears a close resemblance to staphylococcic osteomyelitis occurring in human beings.

FROM AUTHORS' SUMMARY.

NEURON DEGENERATION IN VITAMIN DEFICIENCY. M. M. WINTROBE, D. M. MITCHELL and L. C. KOLB, J. Exper. Med. 68:207, 1938.

Young pigs were given an artificial diet which was presumably adequate in all respects. As they developed, the quantity of yeast was gradually reduced while thiamin (vitamin B) and riboflavin were given in its place. The rate of

growth of the animals decreased, their general condition became impaired, and marked ataxia without motor weakness developed. Histologically the posterior columns of the spinal cord, the dorsal root ganglion cells and the peripheral nerves showed severe degeneration.

FROM AUTHORS' SUMMARY.

FUNCTIONAL HYPERPLASIA OF THE PARATHYROIDS. A. B. EISLER, *Brit. J. Exper. Path.* **19**:342, 1938.

Data are presented which show that significant hyperplasia of the parathyroid glands occurs without concomitant renal damages in rabbits with toxic anemia. The cause of this hyperplasia is discussed with respect to the relation between calcium metabolism and detoxication.

FROM AUTHOR'S SUMMARY.

ORGAN HYPERTROPHY IN THYROID-FED RATS. O. L. V. S. DE WISSELOW and W. J. GRIFFITHS, *Brit. J. Exper. Path.* **19**:347, 1938.

The adrenal medulla is not concerned with hypertrophy of the heart and other organs of rats fed desiccated thyroid substance. The drug 2,4-dinitrophenol, which increases the rate of metabolism of the tissue in general, does not produce hypertrophy of the organs of the rat. Since increasing the rate of the consumption of oxygen by the tissues does not of itself result in cardiac hypertrophy, the effect of thyroid on the heart cannot be ascribed to this aspect of its function. The increase in metabolism and the tachycardia, although they develop simultaneously under the action of thyroid, are unrelated. It is probable that the hypertrophy of the heart is secondary to the increase in the rate of contraction and the extra work performed.

FROM AUTHORS' SUMMARY.

Pathologic Anatomy

INFARCTION OF THE HEART. W. B. BEAN, *Am. Heart J.* **14**:684, 1937.

This is a study of 300 necropsies which disclosed infarction of the heart, selected from 9,626 consecutive necropsies in the Boston City Hospital in the years from 1906 to 1936. Among the patients there was a predominance of males—69.7 per cent. The average ages were 60.1 for males and 61.7 for females. The seventh decade of life showed the largest incidence of infarcts. The first attack was recorded at an earlier age in the case of males than in that of females. A family tendency toward cardiovascular disease and hypertension appeared as a contributory factor; obesity seemed also to be contributory, though thinness did not protect against infarction. Diabetes and disease of the gallbladder were factors, but merely by virtue of the arteriosclerosis with which they were invariably associated. Alcohol and tobacco did not seem etiologically important. The incidence of infarction was significantly low during the summer. In the presence of disease of the coronary arteries a surgical or medical shock acted as a precipitant of coronary thrombosis.

I. DAVIDSON.

INCIDENCE OF MYOCARDIAL INFARCTION WITHOUT PAIN IN TWO HUNDRED AUTOPSED CASES. J. A. KENNEDY, *Am. Heart J.* **14**:703, 1937.

In 4 per cent of the cases of myocardial infarct occurring within eight weeks before death, carefully taken histories failed to reveal any sensation of pain in relation to the infarct. Among old infarcts from which the patients recovered, the number of painless ones was larger (about 22 per cent). Kennedy emphasizes that in about one third of the cases of infarct recorded the circumstances were such that it was impossible to elicit a history of pain and that therefore such infarcts must be excluded.

I. DAVIDSON.

ENDOMETRIUM IN PREGNANCY. A. C. BRODERS and J. R. McDONALD, *Am. J. Clin. Path.* 8:547, 1938.

During the early part of pregnancy the endometrium usually presents a picture more or less comparable to that of the late differentiative phase of the menstrual cycle. This picture is less common in extrauterine pregnancy. The changes in the endometrial glands ("glands of pregnancy") are present in a large proportion of cases of early uterine pregnancy and in a small proportion of cases of extrauterine pregnancy. Occasionally they are seen in the agravid uterus immediately prior to the menstrual period. "The glands of pregnancy" have the same significance as decidual tissue. Decidual tissue was found in the uterus in 5 of 27 cases of extrauterine pregnancy.

FROM AUTHORS' SUMMARY.

STRUCTURE OF THE FILUM TERMINALE. I. M. TARLOV, *Arch. Neurol. & Psychiat.* 40:1, 1938.

The filum terminale is a slender band connecting the last two segments of the spinal cord, i. e. the conus medullaris, with the coccyx. The dura follows the filum down to the second sacral vertebra. The intradural portion of the filum has a definite nerve structure, containing gray matter (small and large multipolar cells), a central canal and nerve fibers. The gray matter cannot be differentiated from the white substance, both containing numerous ependymal cells and also oligodendrocytes and astrocytes. In the extradural portion of the filum the microglia and neuroglia are replaced by Schwann and endoneurial cells. The nerve fibers of the filum run within its connective tissue sheath and are invested with endoneurial and Schwann cells and form bundles. In an adult film products of degeneration occur in the form of corpora amylacea, which are reticulin balls derived from the connective tissue of the filum, staining reddish with Rio Hortega's lithium-silver carbonate.

G. B. HASSIN.

THE THECA INTERNA CONE AND ITS ROLE IN OVULATION. E. O. STRASSMAN, *Surg., Gynec. & Obst.* 67: 299, 1938.

Ovulation is a mechanical process stimulated by the endocrine glands. Its mechanism can be understood only by determining how the graafian follicle reaches the surface of the ovary. A study based on more than 18,000 serial sections of ovaries from human and other mammals, including Carnivora (rat and dog), Rodentia (rabbit) and Ungulata (swine, cow, horse), has demonstrated eccentric growth of the theca interna of the growing follicle. This one-sided proliferation of the theca interna is always directed toward the surface of the ovary, forming a cone, wedge shaped on the cut surface and infiltrating and penetrating the surrounding tissues, thus making a path for the growing follicle. The maturing follicle ascends to the surface of the ovary by following the line of least resistance which is provided by the cone of the theca interna. The average distance between follicle and ovarian surface becomes shorter with the appearance of the thecal proliferation. In human and mammalian ovaries, which have much free ovarian surface, the axis of the thecal cone is directed toward the nearest point on the surface of the ovary. In horses, in which the surface of the ovary is surrounded by connective tissue (mesovarium), the axis of the cone of the theca interna is directed toward the only free spot on the surface of the gland, namely, the ovulatory fossa. The wedge-shaped cone can be demonstrated only in the sections which run through the apex of the cone perpendicularly toward the ovarian surface. A more or less marked degree of edema is present in the surrounding tissues, which facilitates the mechanical progress of the ascending follicle.

FROM AUTHOR'S SUMMARY (WARREN C. HUNTER).

A HISTOLOGICAL STUDY OF THE MUMMY OF HAR-MOSĚ. A. F. B. SHAW, J. Path. & Bact. **47**:115, 1938.

An account is given of the mummy of Har-mosĚ, the lute player and singer of the eighteenth dynasty, who died about 1490 B. C. The organs (lung, liver, gall-bladder, mesentery and intestines) found in the canopic chest were in a remarkably good state of preservation and superior in this respect to any mummified tissues hitherto described from Egypt. The reasons for this were not determined. It was found that the supporting tissues of the organs, especially collagen, cartilage matrix, elastic fibers and argyrophil reticulum, were preserved almost in their original state. For this reason stains which differentiate these elements gave the best results. Although the cytoplasm of unstriated muscle, liver cells and fat cells and of the epithelium of the bronchi, intestines and gallbladder had survived, the nuclei had disappeared. There were no cells in the parenchyma of the lung or in the pleura. The blood vessels sometimes contained material giving the staining reactions of hemoglobin, but erythrocytes and leukocytes were never seen. From the distribution and amount of adipose tissue in the mesentery and around the gallbladder it seems probable that Har-mosĚ was a fat man or at least well nourished. Pathologically the deceased Egyptian showed pulmonary anthracosis and emphysema, an old pleural adhesion, intimal thickening of the superior mesenteric artery and fatty involution of the pulmonary lymph nodes. The two latter lesions may have been due to his age. Evidence is produced in support of the view that Har-mosĚ probably suffered from acute bronchopneumonia and pleurisy, which from their extent must have been the immediate cause of his death.

FROM AUTHOR'S SUMMARY.

ACIDOPHILIC CELL "INCLUSIONS" IN NERVE TISSUE AND IN KIDNEYS OF "NORMAL" PIGEONS. S. NICOLAU and L. KOPCOWSKA, Ann. Inst. Pasteur **60**:308, 1938.

Three varieties of strongly eosinophilic bodies similar to inclusion bodies have been found in the nerve cells of "normal" pigeons." These bodies varied from minute granules to round or oval structures from 3 to 4 microns in diameter and occurred both singly and in groups. Irregular masses of granules were found in the cytoplasm of brain cells. In the nuclei of spinal ganglion cells bodies were seen which often showed an internal structure similar to that of Borna's inclusions. Cytoplasmic inclusions with a complex internal structure were found in the spinal ganglions.

Both cytoplasmic and nuclear inclusions were present in the cortex of the kidney, the nuclear ones being the more frequent. The bodies were rounded refractile structures from 1 to 5 microns in diameter.

Some birds showed only one variety of inclusion, but in others all of these forms were seen. In some of the pigeons interstitial infiltration and perivascularitis of nerve tissue were observed, but inclusions were also present in birds without other histologic changes.

There may be many unknown viruses which do not produce clinical symptoms but only histologic changes and inapparent, i. e. latent, infection.

J. B. GUNNISON.

MYOCARDIAL CHANGES IN DIPHTHERIA. L. OHEIM, Beitr. z. path. Anat. u. z. allg. Path. **100**:195, 1938.

A morphologic study of the myocardium in 50 fatal cases of diphtheria in which there were no complicating infections or other complicating conditions revealed the following successive changes: interstitial edema, myolysis, waxy degeneration, calcification, fatty degeneration, proliferation of fixed cells in the spaces arising from muscle cell degeneration, and leukocytic and lymphocytic infiltrations. Interstitial edema and initial myolysis appeared first on the second to the fourth day of the disease, while the other changes reached their height of intensity from the seventh to the thirteenth day. Reparation by connective tissue proliferation had

commenced by the tenth day. The most severe lesions were encountered in the subendocardial and ring muscle layers of the myocardium, especially of that of the right ventricle. The significance of this distribution in respect to the efficacy of intravenous therapy is discussed.

R. J. LEBOWICH.

SCLEROSIS OF ARTERIOLES OF THE SPLEEN AND KIDNEY IN RELATION TO ARTERIAL HYPERTENSION. K. SCRIBA, *Virchows Arch. f. path. Anat.* **301**:321, 1938.

This is a contribution from Fahr's institute on the still unsolved problem of the interrelationship of arteriosclerosis and arterial hypertension. It is based on histologic examination of the arterioles of the spleen and kidney in a series of 132 subjects aged from 3 months to 82 years; 56 had not had hypertension during life and 76 had. The presence of hypertension was determined by the readings of blood pressure made during life or by the observation of left ventricular hypertrophy at necropsy. The spleen was included in the investigation because of its functional activity and its reaction to infection, toxemia and other general conditions, factors which Scriba believed might be important in the development of arteriosclerosis. In the first five decades of life arteriosclerosis was as frequent or even more frequent in the spleen and kidney in nonhypertensive persons as in those with hypertension. From the fifth decade on sclerosis of arterioles became progressively more frequent in those who had had hypertension. Scriba interprets his observations as indicating that the etiologic factors in the development of arteriosclerosis are local functional reactions of the organ to mechanical or toxic factors, the constitutional element, various dyscrasias and age. Arterial hypertension is not the cause of arteriosclerosis but is at most only an accessory factor.

O. T. SCHULTZ.

ORIGIN OF THE INTRAEPITHELIAL HYALINE DROPLETS OF THE KIDNEY. A. HEIN, *Virchows Arch. f. path. Anat.* **301**:339, 1938.

To determine the origin and significance of the hyaline droplets of the epithelium of the main convoluted tubules of the kidney which are observed in association with albuminuria, Hein injected solutions of proteins into the abdominal cavity of the salamander. Human blood serum and its fractions and solution of egg white were used in varying amounts and over varying periods. Hyaline droplets of albuminous material appeared in the convoluted tubular epithelium. A prerequisite for their presence was the presence of protein in the lumen of the tubules, arrived there through the open nephrons in the case of proteins with large molecules and through the glomeruli in the case of molecules of smaller size. The droplets were formed in the reabsorption by the tubular epithelium of the protein from the lumen. The process was one of temporary storage of absorbed material. A certain amount of protein injected over a short period caused a more marked formation of droplets than the same quantity administered over a longer period of days. Albumose derived from commercial peptone also caused the formation of hyaline droplets. Cessation of the administration of proteins was followed by disappearance of the droplets. The tubular epithelium may utilize or break down the stored absorbed protein.

O. T. SCHULTZ.

THE VASCULAR CHANGES IN VARIOUS TYPES OF PULMONARY TUBERCULOSIS. B. NEUBERT, *Virchows Arch. f. path. Anat.* **301**:364, 1938.

The types of pulmonary tuberculosis selected for investigation were the primary focus, caseous pneumonia, bronchogenic aspiration tuberculosis, lymphogenous productive tuberculosis, hematogenous spread, giving rise to miliary tuberculosis, and cavernous tuberculosis. The vascular reaction may be specific or nonspecific. In the latter, a diffuse proliferative process leads to partial or complete obliteration of the blood vessel. In each type of tuberculosis the character of the vas-

cular reaction was noted, whether specific or nonspecific, and the number of vessels in the tuberculous area and in the adjacent parenchyma revealing partial (one fourth) to complete obliteration was tabulated. Obliterated vessels were most numerous in the primary focus and least numerous in the hematogenous form of spread. In general, obliterated vessels were more numerous in fairly rapidly progressing lesions than in more slowly progressing ones, except that caseous pneumonia may progress so rapidly that there is not time for an obliterating reaction on the part of the vessels. Nonspecific obliteration in the peripheral zone of nontuberculous inflammation precedes the advancing spread of the parenchymatous process. The character of the parenchymatous involvement is not dependent on the vascular change, but the latter may hasten the progress of tissue destruction.

O. T. SCHULTZ.

CORRELATION OF FETAL BODY LENGTH WITH THE WEIGHTS OF ORGANS. A. GIORDANO, *Virchows Arch. f. path. Anat.* **301**:380, 1938.

This is a correlation of body length with the weights of the heart, lungs, liver, spleen and kidneys, according to accepted current methods of statistical analysis. This conclusion is based on a study of 445 infants, dead at birth or shortly after birth, and varying in length from 30 to 67 cm. The females in the series numbered 199 and the males 246. A striking feature of this series is that 25 per cent of the infants had a body length of from 52 to 67 cm., indicating a duration of pregnancy beyond the normal limit. The high mortality in this group was due to intracranial hemorrhage. It suggests the advisability of terminating pregnancy at the normal term. The weights of the organs in the various length groups are expressed in tables and graphs. In the length groups 40 to 47 cm. and 47 to 52 cm. the organ weights were practically the same for both males and females. In both males and females the rate of increase in organ weights was greater at body lengths of from 47 to 52 cm. than in younger fetuses with lesser body lengths. In the length group 52 to 67 cm., representing a prolonged duration of pregnancy, organ weights increased somewhat less rapidly, but the organ weights of males were decidedly greater than those of females in this group.

O. T. SCHULTZ.

Pathologic Chemistry and Physics

BLOOD PLASMA PROTEINS AND LIVER INJURY. C. C. ERICKSON, G. P. HECKEL and R. E. KNUTTI, *Am. J. Path.* **14**:537, 1938.

By frequent oral administration of carbon tetrachloride to dogs it has been possible to produce moderate cirrhotic changes in their livers. In such animals the plasma protein concentration falls slightly, and this fall appears to be due largely to loss of albumin. Continued oral administration of acacia combined with carbon tetrachloride results in deposition of what appears to be acacia in hepatic cells, as in the case of administration of acacia alone. Other sites of deposition of acacia in dogs receiving carbon tetrachloride and acacia appear to be the sinusoidal lining cells of the spleen and the large mononuclear phagocytic cells which have been found in sparse numbers in the lungs, spleen, lymph nodes and bone marrow. Clinically these animals remain in remarkably good condition, and, although the concentration of their plasma protein may be well below the edema level, there has been no evidence of edema. The bleeding time of such dogs is prolonged, and this is thought to be associated with low concentration of fibrinogen, although the possibility of a scanty supply of prothrombin must also be considered. The diminution of the concentration of plasma protein is somewhat more marked in animals receiving carbon tetrachloride and acacia than in those receiving either of these substances alone. This is particularly true of changes in the concentration of fibrinogen. The reactions of albumin, globulin and fibrinogen to various

types of hepatic injury indicate that these substances may be produced independently of each other and that the liver is concerned in their production.

FROM AUTHORS' SUMMARY.

p_H IN PNEUMOCOCCUS EXUDATES. W. H. KELLEY, E. N. SCADRON and B. M. SHINNERS, *J. Exper. Med.* **67**:659, 1938.

The hydrogen ion concentration in the lesions of experimental pneumococcic infection has been estimated directly by determinations of p_H on exudates from living animals. For indirect evidence of an increase in hydrogen ion concentration within the lesions, the difference in sugar content between exudate and blood from animals with pneumococcic infection has been measured. With sanguineous exudate from the consolidated lungs of dogs with experimental pneumococcic pneumonia, the findings were not always consistent, but usually there was either direct or indirect evidence of increased hydrogen ion concentration. The physicochemical changes in exudate from animals treated with artificial pneumothorax showed no important differences from those in other specimens. In concurrence with Lord's observation of increased acidity of pneumonic exudate obtained at autopsy, sugar concentrations, which are low in the blood, were markedly reduced in exudates from animals which had died of the infection. Serous exudates from dermal pneumococcic infection in rabbits uniformly showed definite acidity by both direct and indirect methods of estimation. The hydrogen ion concentration in exudate from dermal pneumococcic infection in rabbits varied between p_H 6.87 and 6.66 but was not always proportional to the difference in sugar concentration between the exudate and the blood. While the hydrogen ion concentration of pneumonic exudate from rabbits is similar to that attained in the pneumonic exudate from dogs, it is of lesser magnitude than that which Takahashi has described in the pus of empyema secondary to infection with *Pneumococcus*.

FROM AUTHORS' SUMMARY.

DEMONSTRATION OF PLASMA ANTICOAGULANT IN EXUDATES OF BACTERIAL ORIGIN. E. NETER, *J. Infect. Dis.* **63**:193, 1938.

Exudates of bacterial origin may inhibit the coagulation of human plasma. The purulent exudates tested comprised human spinal, empyemic and peritoneal fluids as well as the contents of abscesses. The micro-organisms recovered from such anticoagulating exudates were *Streptococcus haemolyticus*, *Streptococcus faecalis*, *Staphylococcus*, *Pneumococcus*, *Haemophilus influenzae*, *Bacillus coli* and *Clostridium welchii*.

Human pneumococcic exudates contained anticoagulant less frequently than those in which the enterococcus was present. Exudates due to the hemolytic streptococcus may contain either anticoagulant or fibrinolysin or both—or such exudates may lack fibrinolytic as well as anticoagulating properties.

FROM AUTHOR'S SUMMARY.

LAW OF CHEMICAL EQUILIBRIUM IN BIOLOGY. F. C. McLEAN, *Physiol. Rev.* **18**:495, 1938.

So far as McLean is aware, this is the first attempt to review the contributions of the law of chemical equilibrium to the study of biologic problems. It might be thought a priori that this law, being a statement of the conditions in a chemical system at equilibrium, might be of very limited application to biologic processes. On the contrary, its role in the elucidation of the mechanisms of the acid-base balance and in the discovery of physiologically important properties of hemoglobin can hardly be overestimated, and it has opened the way for equally significant progress in the understanding of the complexities of intermediary metabolism. It should be increasingly useful as biology becomes more and more concerned with the analysis of chemical processes in the living organism.

FROM AUTHOR'S CONCLUSION.

A SPECIAL FORM OF ERYTHROCYTE POSSESSING INCREASED RESISTANCE TO HYPOTONIC SALINE. A. M. BARRETT, J. Path. & Bact. **46**:603, 1938.

A special morphologic type of red corpuscle is described which is constantly associated with the presence in the blood of an increased proportion of corpuscles resistant to 0.3 per cent sodium chloride solution. Evidence is given that corpuscles of this type themselves possess increased resistance to hypotonic saline solution. In stained dry films such corpuscles have a characteristic appearance to which Barrett has applied the term "target corpuscle"; in wet films, however, these corpuscles are bowl shaped. In a solution of formaldehyde the corpuscles assumed the target appearance, and it was thus possible to observe the actual shape of the target corpuscle. The correctness of the conclusions regarding the actual shape of the cells in dry and in wet films is shown by roentgen photographs of plasticine models. Evidence is given that in blood containing cells of this type the red corpuscles are abnormally thin. The relationship between the shape of erythrocytes and their resistance to hypotonic salt solution is discussed.

FROM AUTHOR'S SUMMARY.

HISTOCHEMISTRY OF THE ADRENAL MEDULLA. F. SCHULTZ, Beitr. z. path. Anat. u. z. allg. Path. **101**:32, 1938.

In frozen sections of formaldehyde-fixed adrenal medulla from horses, cattle, goats, sheep and hogs it is possible to demonstrate, by means of a stain composed of thionine and tartaric acid, a metachromatic yellow-staining substance of lipoid character. The reaction may be an oxidation of some lipoid-like substance. The yellow coloration is brought out by 95 per cent alcohol and is most intense in adrenal medulla from horses and cattle and less marked in that from hogs, sheep and goats. A similar substance was shown to be present in human adrenal medulla by Feyrtet in 1936.

R. J. LEBOWICH.

Microbiology and Parasitology

THE INCIDENCE OF EXTRAPULMONARY PRIMARY TUBERCULOSIS. H. C. SWEANY and W. L. M. MARTINSEN, Am. Rev. Tuberc. **37**:465, 1938.

The records of 132 autopsies were reviewed with attention being paid to calcified foci to determine the percentage of infections from each portal of entry. The lungs and hilar lymph nodes were involved in 73.5 per cent of isolated primary infections, or in 79 per cent of infections if one adds those in which there were multiple portals of entry. The gastrointestinal tract was the portal for 8.3 per cent of isolated infections and for 12.9 per cent of infections if one adds those in which there were multiple portals of entry. The cervical or head region was the portal for 1.8 per cent of isolated primary infections and for 4.5 per cent of infections if one adds those with multiple portals of entry. Three (2.3 per cent) of the primary infections were pleural infections. In three (3.3 per cent) of the cases "cryptic" lesions occurred in the liver or lymph nodes with no demonstrable local lesions. There were 3 (2.3 per cent) cases in which the portal was doubtful and 7 (5.3 per cent) in which no calcifications were found. (In 71 cases no roentgenograms of the neck were made.) In 30.6 per cent of the cases primary hematogenous calcifications were observed in the liver and spleen.

H. J. CORPER.

COCCIDIOIDOMYCOSIS: THE PRELIMINARY ACUTE INFECTION WITH FUNGUS COCCIDIOIDES. ERNEST C. DICKSON, J. A. M. A. **111**:1362, 1938.

The preliminary illness caused by infection with the fungus coccidioides has been recognized. The disease is caused by inhalation of the chlamydo-spores, which are formed in the vegetative phase of growth of the fungus. It is a form of infection of the respiratory tract, in many cases accompanied by erythema nodosum, and the great majority of patients recover promptly without complications. The

incidence of erythema nodosum is very high but not constant. When this condition occurs the disease is known in the San Joaquin Valley as "valley fever" or "desert fever." The acute illness, whether or not there is erythema nodosum, may progress to coccidioidal granuloma.

FROM AUTHOR'S SUMMARY.

VIRUS OF MENINGITIS AND PNEUMONITIS. T. FRANCIS JR. and T. P. MAGILL, *J. Exper. Med.* **68**:147, 1938.

An infectious agent is described which belongs apparently to the class of filtrable viruses but which on the basis of the evidence at hand is not to be identified with any virus previously described. It has multiple tropisms and is pathogenic for mice, for ferrets and for monkeys of the species *Macacus rhesus* and *Macacus cynomolgus*. Mice and ferrets infected intranasally show extensive pneumonic lesions of fatal severity. Monkeys inoculated intracerebrally show lymphocytic choriomeningitis, from which they recover, while mice similarly inoculated show rapidly fatal choriomeningitis. Mice which receive the virus by intraperitoneal or subcutaneous routes show fatal paralysis in a moderate proportion of their number, while the remainder become immune according to the intracerebral test but not according to the intranasal test. In mice, monkeys, ferrets, rabbits and guinea pigs subcutaneous inoculation causes local granulomatous induration of the skin with enlargement of the regional lymph nodes. In 1936 ferrets inoculated with throat washings of patients suffering from an epidemic disease clinically indistinguishable from epidemic influenza yielded the virus repeatedly. It is impossible, however, to draw any conclusion as to whether the virus has its origin in ferrets or in man. Although the new agent possesses many features in common with the virus of lymphocytic choriomeningitis and the virus of lymphogranuloma venereum, cross immunity tests have failed to yield any evidence that it is immunologically related to either of these viruses. For purposes of identification, the name "virus of acute meningopneumonitis" is suggested.

FROM AUTHORS' SUMMARY.

VIRUS OF EQUINE ENCEPHALOMYELITIS. P. K. OLITSKY and C. G. HARFORD, *J. Exper. Med.* **68**:173, 1938.

Young (12 to 15 day old) mice are approximately as susceptible to the virus of equine encephalomyelitis, Eastern or Western strain, when it is given intraperitoneally as are adult mice when the virus is injected intracerebrally. With this susceptibility to the virus injected by the intraperitoneal route as a basis, intraperitoneal injection of immune serum-virus mixtures was found to result in protection in dilutions which give rise to infection after intracerebral inoculation. The difference in protection by the two indicated routes was shown not to depend on the amount of inoculum or on the age of the mice given intracerebral injections. Incubation at 37 C. for two and one-half hours neither increases nor diminishes the protective action of immune serum when the intraperitoneal method is employed. The phenomenon of selective protection in different tissues is elicited by the serums of hyperimmunized mice, guinea pigs and rabbits and by serums derived from horses infected with the disease in nature or exposed to it by contact with infected animals. Of 4 horses recovered from the malady, all showed antibody in their serum; of 9 others exposed by contact, 4 revealed antiviral bodies when the intraperitoneal technic was employed. These tests on horse serum have pointed to the potential value of this procedure for epidemiologic studies. Finally, the reaction itself has significance through its bearing on the mechanism of immunity.

FROM AUTHORS' SUMMARY.

SMALL COLONY VARIATION IN *B. PARATYPHOSIS B* (Tidy). A. HADDOW, *J. Infect. Dis.* **63**:129, 1938.

A description is given of the spontaneous occurrence of dwarf colony variation in a laboratory strain of *Bacillus paratyphosus B* (Tidy). While the original

variant bore an obvious relation to the normal form of the parent culture, it subsequently yielded a minute colony containing gram-negative coccal forms similar to the G type described by Hadley for *Bacterium dysenteriae* Shiga and other bacterial species. The biologic characters of this variant are given in detail, and evidence is presented in support of Hadley's contention that such colonies contain filtrable elements. No reversion was obtained, but in the course of propagation over four years the G type gave rise to a number of closely related discontinuous variants, whose features are described. The mode of origin and the nature of the G type in relation to the parent organism are discussed with other cognate topics.

FROM AUTHOR'S SUMMARY.

GROWTH OF *PROTEUS*. P. FILDES, Brit. J. Exper. Path. **19**:239, 1938.

Nicotinic acid is an essential accessory nutrient for 10 strains of *Proteus*. It is the only nitrogenous substance required in metabolism which cannot be synthesized from ammonia (NH_3). Its function is to supply material for the synthesis of pyridine nucleotides.

FROM AUTHOR'S SUMMARY.

DARK-GROUND STUDIES OF FLAGELLAR AND SOMATIC AGGLUTINATION OF *B. TYPHOSUS*. A. PIJPER, J. Path. & Bact. **47**:1, 1938.

An account is given of dark ground methods, with the sun being used as the source of light, by means of which the flagella of *Bacillus typhosus* and similar bacteria swimming in broth can be seen and photographed. *B. typhosus* and similar bacteria swim by means of a long tail. At rest, the tail unwinds itself into two rather broadly coiled spiral flagella, which are attached somewhere near the middle of the body of the bacillus and take up a position at an angle to its long axis. When resuming activity, the two flagella start revolving round their own axis, stretch, and become twisted round each other at the rear end of the bacillus, where they form the tail. Each flagellum consists of a number of extremely thin threads, as can be seen when the flagella finally disintegrate. The drying up of a drop of bacterial suspension, such as precedes staining, produces artefacts in the flagellar equipment of bacilli. In so-called H agglutination the actual process is merely a secondary nonspecific mechanical event. The specific phase of H agglutination is that in which the tails and flagella become covered with a granular deposit which finally completely covers and ensheathes them. The resulting thickened and stiff spiral structures cannot escape becoming entangled, and so the bacilli become attached to one another. This is true both for live bacilli and for formaldehydized suspended bacilli. In O agglutination the agglutination is the primary event. Here one can see that the bacilli exert mutual attraction. The force acts in the direction of the long axis of the bacillus which joins with its fellow end to end. This leads to building up of clumps which exhibit a regular pattern. Neither tails nor flagella take part, but their motility and appearance are affected.

FROM AUTHOR'S SUMMARY.

INFECTIOUS ANEMIA OF HORSES. L. BALOZET, Arch. Inst. Pasteur de Tunis **27**: 189, 1938.

The virus of infectious anemia of horses was rendered harmless by treatment with 0.1 per cent sodium ricinoleate. Inoculation of such inactivated virus did not produce immunity in the single donkey treated. Small doses of virus produced only a mild infection in the first donkey inoculated, but in another animal the injection of similar doses resulted in death. Virus mixed with hydrous wool fat and olive oil likewise produced a mild infection in the first animal inoculated and a fatal one in a second animal. Hence attempts to produce an attenuated form of the disease by the use of small doses or by incorporating the virus in a substance tending to prevent its rapid absorption were unsuccessful.

FROM AUTHOR'S SUMMARY.

Immunology

SENSITIZATION WITH HEAT-KILLED TUBERCLE BACILLI. J. FREUND and E. I. OPIE, *J. Exper. Med.* **68**:273, 1938.

An intracutaneous injection of a small quantity of heat-killed tubercle bacilli into a previously normal animal produces a nodule which persists from eight to twelve weeks; an injection of the same amount into a well sensitized animal produces a lesion which ulcerates within from one to three weeks and is completely healed after about five weeks. Complete healing is functional evidence of the disappearance of the antigen. An intracutaneous injection of heat-killed tubercle bacilli induces more rapid sensitization than a subcutaneous or an intravenous injection, but after repeated injections the difference disappears. An increase in the quantities of heat-killed tubercle bacilli injected or division of the quantity among several simultaneous injections will accelerate sensitization. The rapidity of antibody formation as measured by complement fixation varies in different rabbits under the same conditions, but complement fixation is always demonstrable after repeated injections of heat-killed tubercle bacilli. Antibody formation is more rapid and reaches higher titers with intravenous than with intracutaneous or with subcutaneous injections. It is accelerated by division of the injected antigen among multiple simultaneous injections. Small quantities of BCG induce rapid sensitization and more abundant antibody formation, measured by complement fixation, than the same amounts of heat-killed tubercle bacilli, but with repeated injections the difference disappears. Animals that are sensitized and immunized (allergic) before infection are in most instances more resistant to infection than previously normal animals, but there is no correlation between the intensity of sensitization or the titer of antibodies, on the one hand, and the resistance to infection, on the other. It is probable that the skin test for sensitization and complement fixation as applied to the blood serum measure antibodies or other factors determining sensitization and immunity that are in excess of those actively concerned in the maintenance of resistance.

FROM AUTHORS' SUMMARY.

ANTIGENIC STRUCTURE OF SPERMATOOZOA. W. HENLE, G. HENLE and L. A. CHAMBERS, *J. Exper. Med.* **68**:335, 1938.

By means of the absorption technic as applied to homologous spermatozoal serums, a head-specific and a tail-specific antigen could be demonstrated. Both are heat labile. A heat-stable antigen was found to be common to both the head and the tail. This substance is species specific. Antibodies against the head-specific and the tail-specific antigen led to two different types of agglutination as shown by the slide method. Using heterologous antisera against spermatozoa, three different cross-reacting antigens could be observed, two in the head and one in the tail. One of the head antigens is not active in the native cell; it comes to action only after breaking of the cell. Antibodies against this substance were not found in antisera against native bull spermatozoa but were formed when vibrated spermatozoa or heads were injected into rabbits. The cross reactions can be removed from an antiserum leaving the head-specific as well as the tail-specific reaction intact.

FROM AUTHORS' SUMMARY.

INACTIVATION OF TETANUS TOXIN BY CRYSTALLINE VITAMIN C (l-ASCORBIC ACID). C. W. JUNGBLUT, *J. Immunol.* **33**:203, 1937.

Three preparations of vitamin C (l-ascorbic acid)—a natural crystalline extract, a stable synthetic solution and a crystalline synthetic product—were capable of inactivating 2 minimal lethal doses of tetanus toxin in the test tube but not in vivo within the pH range of from 6.1 to 6.4 and in quantities varying

from 1 to 10 mg. The inactivation was not due to the chemical reaction, and it differed from the toxin-antitoxin reactions, because it did not follow the laws of multiple proportions.

I. DAVIDSOHN.

EXPERIMENTAL HYPERSENSITIVENESS IN THE RHESUS MONKEY. H. W. STRAUS and A. F. Coca, *J. Immunol.* **33**:215, 1937.

Induced sensitivity to poison ivy in rhesus monkeys was limited to the isolated portion of the skin of the arm, or to the remainder of the body's surface after an encircling incision of the skin of the arm and dissection downward had interrupted the continuity of the skin. The conclusion is drawn that the sensitivity of contact dermatitis is developed locally in the cells of the epidermis and that the agents that produce hypersensitiveness spread through the oily substances of the skin by continuity and not through the fluids of the body.

I. DAVIDSOHN.

HETEROGENETIC HEMAGGLUTININS IN MAN FOLLOWING THERAPEUTIC INJECTIONS OF IMMUNE SERUM FROM RABBITS. F. SCHIFF, *J. Immunol.* **33**:305, 1937.

The serums of some patients who had been given injections of antipneumococcus type-specific rabbit immune serums showed rises in agglutinins for the red blood cells of the rabbit, sheep, ox, horse and rhesus monkey. These antibodies behaved much as do the agglutinins that appear following injections of horse serum. The agglutinins for sheep red cells were more readily removed by the red cells of all the species, and, on the other hand, the red cells of the rabbit demonstrated the greatest capacity for absorbing the different hemagglutinins. The antibodies that develop in response to horse and rabbit serum behave differently from those that develop in the serums of patients with infectious mononucleosis. Schiff suggests the term "serum sickness antigen" for the common antigenic substance in the serums of the horse and rabbit and in the red cells of horse, rabbit, sheep and ox. It is heat stable; it has an alcohol-soluble fraction, and it has a water-soluble fraction that can be removed from the alcohol-treated beef cells.

I. DAVIDSOHN.

AGGREGATION OF ANTIBODY-ANTIGEN COMPOUNDS. J. T. DUNCAN, *Brit. J. Exper. Path.* **19**:328, 1938.

The view that the aggregation of the immune compound is specific in character, as demanded by the lattice hypothesis, is supported by the results of agglutination reactions with immune serum, and, although the effect of a nonspecific factor in aggregation was never absent from the precipitation reactions, its incidence seems to be secondary to a specific combination, and the results of these tests may be accepted as not inconsistent with the lattice hypothesis.

FROM AUTHOR'S SUMMARY.

NATURE OF THE O PROPERTY. L. HIRSZFELD and Z. KOSTUCH, *Schweiz. Ztschr. f. allg. Path. u. Bakt.* **1**:23, 1938.

In order to ascertain the nature of the agglutinin in human O blood, Hirszfeld and Kostuch tested members of 58 families with 138 children by means of anti-Shiga bacillus immune goat serum and by means of normal bovine serum—serums both of which had the peculiarity of agglutinating by preference blood belonging to group O. In this study there was one family in which the parents both belonged to subgroup A₁ and a child to subgroup A₂B; this finding was attributed by the writers to illegitimacy. There were no exceptions to the theory of the inheritance of the subgroups of A and AB by four allelomorphous genes, advanced by Thomsen, Friedenreich and Worsaae. The anti-O serums reacted most strongly with bloods belonging to group O, somewhat less intensely with bloods of subgroups A₂ and A₁B, more weakly with bloods of group B and subgroup A₁ and not at all with

bloods of subgroup A₁B. Hirszfeld and Kostuch believe that the ability of bloods of subgroup A₂ to react with anti-O serums is not due to the effect of the O gene in heterozygous blood of this subgroup but indicates that the agglutinin A₂ represents a less pronounced mutation from agglutinin O than agglutinin A₁. Agglutinin B would hold a place somewhere between A₁ and A₂.

A. S. WIENER.

BLOOD GROUP PROPERTIES IN SHEEP. · T. ANDERSEN, *Ztschr. f. Rassenphysiol.* **10**: 88, 1938.

On the basis of isohemolysis, Andersen was able to confirm the broad classification of sheep blood into three groups, Ro, O anti-R and Oo. When sheep of any group were immunized with blood of other sheep, even those belonging to the same group, immune isohemolysins were readily produced. These immune isoantibodies were qualitatively different from the natural isoantibodies, since they were not absorbable with human A₁ blood. By immunizing rabbits with sheep blood Andersen obtained group-specific hemolysins in about half the rabbits.

A. S. WIENER.

Tumors

CARCINOGENIC ACTION OF METHYL DERIVATIVES OF 1:2-BENZANTHRACENE. M. J. SHEAR, *Am. J. Cancer* **33**:499, 1938.

Of 21 compounds subcutaneously injected into pure strain mice in tests for carcinogenic activity, 10 produced tumors at the sites of injection. Subcutaneous tumors were produced in mice by 5,10-dimethyl-1,2-benzanthracene about as rapidly as by cholanthrene, showing that the pentacyclic system of the latter is not essential for high carcinogenic potency. The production of subcutaneous tumors by 10-methyl-1,2-benzanthracene with the skin-painting technic was slower than the production of subcutaneous tumors with the injection technic. Tumors were produced by 5,9-dimethyl-1,2-benzanthracene about as rapidly as by cholanthrene. The 9-methyl derivative was also found to be a potent carcinogen, but the latent period was longer than with the 5,9-dimethyl derivative. The 4,10-ace derivative was found to be carcinogenic, especially in small doses which did not produce severe local damage of tissue. The 1',2',3',4'-tetrahydro derivative of 4,10-ace-1,2-benzanthracene was also found to be carcinogenic. 20-ethylcholanthrene produced tumors in a high proportion of the mice but acted more slowly than 20-methyl-cholanthrene or cholanthrene. No tumors were produced by *s*-triphenylbenzene even after twenty months.

FROM AUTHOR'S SUMMARY.

GRANULOSA CELL CARCINOMA. E. H. NORRIS, *Am. J. Cancer* **33**:538, 1938.

The granulosa cell carcinoma may be defined as a malignant tumor of the ovary the histologic structure of which commonly and characteristically shows the presence of granulosa-like cells which manifest a tendency to surround more or less typical follicles. The tumor is associated with signs and symptoms which may be ascribed to the influence of excessive amounts of estrogen. The granulosa cell carcinoma may develop in any of the decades of life, and the principal clinical manifestations vary with and depend almost entirely on the age of the patient and on the epoch of the female sexual cycle in which the tumor develops. In children the granulosa cell carcinoma is a cause of precocious puberty. In the older age groups the effects are chiefly concerned with menstrual phenomena. The general course of the disease is continuously progressive, and in untreated patients it leads to death from malignant metastases. Early surgical removal of the primary tumor offers the only hope of permanent relief; in general the operative procedure should be of a radical nature. The postoperative result is good, and if the tumor can be removed the symptoms disappear. The differential diagnosis

is not difficult to make on clinical grounds in children or in women past the menopause but may be impossible to make in women seen during the reproductive epoch. The histologic structure of the granulosa cell carcinoma is variable within wide limits; the pattern varies from typical follicle-like structures, broad epithelial bands and narrow cords to carcinoma-like pictures. There seems to be no advantage in a subdivision of granulosa cell carcinoma on the basis of the different histologic patterns.

FROM AUTHOR'S SUMMARY.

HYPOPHYSEAL TUMORS INDUCED BY ESTROGENIC HORMONE. B. ZONDEK, *Am. J. Cancer* **33**:555, 1938.

Estrogen injected either parenterally or percutaneously into 240 infantile rats caused eunuchoid dwarfing of all of them. Such treatment carried on for four months caused pituitary enlargement in the males; in the females the gland remained grossly normal. After treatment had continued for eight months the pituitaries of 29 of 35 rats, both males and females were transformed into enormous hypophysial tumors. These tumors caused the death of the animals. The duration of treatment was of primary importance, no tumor developing in less than seven months; the size of the dose of estrogen was of only secondary significance. The tumors could be diagnosed by typical clinical symptoms, the most characteristic of which was a decrease in the body temperature. This was shown not to be due to hypoglycemia. The enlarged pituitaries and the tumors contained the same amount of gonadotropic substance as the normal pituitaries of control animals. It was therefore not the production but the utilization of the gonadotropic hormone that was inhibited in the eunuchoid dwarf rats, causing a functional deficiency.

FROM AUTHOR'S SUMMARY.

IMPLANTATION CARCINOMA OF THE TUBAL MUCOSA. J. A. SAMPSON, *Am. J. Path.* **14**:385, 1938.

The pathogenesis, structure, form and life history of carcinomatous implants of ovarian origin on the tubal mucosa are the same as the pathogenesis, structure, form and life history of similar implants on the peritoneal serosa.

FROM AUTHOR'S SUMMARY.

MORPHOLOGICAL VARIATIONS OF TUMOR CELLS. O. SAPHIR, *Am. J. Path.* **14**:443, 1938.

A study of miscellaneous types of carcinoma revealed morphologic variations among the individual tumor cells of particular neoplasms. These morphologic differences were not those always encountered in malignant tumors but were often so heterogeneous as to obscure the exact nature of the tumor. They were principally brought about by the presence of seemingly different types of tumor cells in an individual tumor. Thus, basal cells or transitional cells were found in squamous cell carcinoma. The presence of various types of tumor cells in single tumors may be due to a number of factors, which are discussed. However, the findings presented here seem to indicate that such a tumor arises from an area which a priori contained the various cells of which the tumor consists, or derivatives of these cells. Such morphologic variations must be taken into consideration in the grading of a carcinoma and also in the determination of its sensitivity to radium. The relative resistance to radium shown by basal cell or transitional cell carcinoma in some cases could be ascribed to the presence of many squamous cells in both tumors.

FROM AUTHOR'S SUMMARY.

THE GENETICS OF CANCER IN MICE. J. J. BITTNER, *Quart. Rev. Biol.* **13**:51, 1938.

From the data tabulated during the past ten years it is evident that some progress has been made in comprehending some of the details of the inheritance of susceptibility to some forms of cancer. Not all types of tumors may be grouped

and studied in a single experiment; each type must be considered by itself. The problem is by no means solved, but a start has been made by the use of homozygous stocks of mice, and geneticists display increasing interest in the problem. Much of the controversy is due to the application of genetic principles to data which are not suitable for this type of interpretation.

Application of the findings in animal experimentation to the disease in man is problematic and remains for the future. The impossibility of securing human data comparable with those on inbred strains of animals whose matings may be controlled needs little comment. The reliability of a considerable proportion of the human data is questionable and leaves much to be desired.

FROM AUTHOR'S SUMMARY.

VISCERAL METASTASIS FROM RECTAL CARCINOMA. C. E. BROWN and S. WARREN, Surg., Gynec. & Obst. 66:611, 1938.

The tendency of rectal carcinoma to metastasize via the blood stream varies in general with the degree of differentiation. As a rule, the longer the duration of rectal carcinoma the greater is the number of visceral metastases. Metastasis to bone occurred in 5 per cent of the series. The more the primary growth penetrates the wall of the bowel, the greater are the chances of blood-borne extensions. The reliability of a prognosis of visceral metastases based on observation of local intravascular metastasis increases with the duration of the disease. Only 1 of the 70 persons showing visceral metastases failed to show local intravascular involvement, while one fourth of those whose lymph nodes were free from invasion had visceral deposits. Sections of the primary growth in rectal carcinoma should be scrutinized carefully for invasion of capillaries or veins by tumor cells, because such invasion often means visceral metastases, and its absence, provided at least three sections from different parts of the growth have been examined, nearly always rules out visceral involvement. The efficiency of this sign—invasion of capillaries or veins—in predicting visceral or bone metastases outranks that of neoplastic lymph nodes. FROM AUTHORS' SUMMARY (WARREN C. HUNTER).

INFLAMMATION AND CARCINOGENESIS. P. R. PEACOCK and S. BECK, Brit. J. Exper. Path. 19:315, 1938.

The induction of sarcoma in the connective tissues of mice following injection of 3,4-benzpyrene in various solvents depends more on the rate of absorption of the benzpyrene than on the early local tissue reaction to the solvent. In mice in which benzpyrene is eliminated within three months tumors are rare (5 of 41 surviving for four months); in those in which elimination is delayed beyond six months tumors are common (34 of 46 surviving for four months). Tumors occur at points of optimal concentration of benzpyrene, which in these experiments were found to be a few millimeters away from the principal loci of benzpyrene.

FROM AUTHORS' SUMMARY.

PRECANCEROUS SKIN CHANGES. J. W. ORR, J. Path. & Bact. 46:495, 1938.

An investigation has been made of the changes in the skin of white mice treated with six carcinogenic hydrocarbons, ten noncarcinogenic hydrocarbons of related chemical structure and a group of unrelated irritants. These substances have been used in solution in benzene and acetone, the latter solvent being of greater value for the present purpose, as it is without effect on normal mouse skin. Early epilation is a striking feature of the action of cholanthrene, methylcholanthrene, 3,4-benzpyrene and 1,2,4,6-dibenzanthracene but not of 1,2,5,6-dibenzacridine and 3,4-benzphenanthrene. It occurs with some noncarcinogenic hydrocarbons but to a much less extent than with the four most potent carcinogens. After epilation by a carcinogen, the regenerated hair is abnormal. The appearance of tumors is preceded by progressive thickening and loss of elasticity and

by passive congestion of the skin. Ulceration of the surface does not bear any relationship to tumor formation. Histologically, the carcinogens produce squamous hyperplasia of the epidermis in the first weeks of treatment. Thereafter, the progressive changes are found in the deeper tissues, and the most significant phenomena are: (1) transformation of the collagen of the superficial, and later of the whole, dermis into a fine-fibered, nonrefractile type; (2) passive congestion of the subcutis; (3) alterations in the texture of the elastic tissue without necessarily an increase in its amount, and (4) absence of inflammatory cell infiltration. When tumors appear, they are frequently related to fibrous scars in the subcutis, to the gaps in the elastic tissue of the dermis or to both. In comparing the carcinogens it was observed that the rate of progress of the changes cited corresponds with the rapidity with which tumors are induced. Similarly, mice in which tumors appear early or late are those in which these changes advance rapidly or slowly, respectively. When comparable changes occur with noncarcinogenic applications, evidence of inflammation is usually present. It is suggested that the cancerization of the epithelial cells by the carcinogenic hydrocarbons is at any rate partly the result of the changes in the deeper tissues and that its mechanism may be related to the consequent interference with their nutrition.

FROM AUTHOR'S SUMMARY.

MESOTHELIOMA OF THE PLEURA. L. CORNIL, V. AUDIBERT, L. MONTEL and M. MOSINGER, *Bull. Assoc. franç. p. l'étude du cancer* **27**:51, 1938.

The article contains a comprehensive 40 page review of the subject of primary tumors of the pleura. Two cases, one of primary mesothelioma, the other of secondary carcinoma, are reported. The secondary tumor presented diagnostic difficulties, as the primary tumor could not be found. The squamous cell structure of the growth, which covered the entire pleura, suggested that the primary tumor was in the periphery of the lung. The other tumor occurred in a 31 year old man with a serofibrinous and later a hemorrhagic pleural effusion, with fever and with loss of weight of eight months' duration. Twenty-one months after the onset a nodule developed in the wall of the chest. It had the structure of a sarcoma. At necropsy the whole lung was encased in a shell from 25 to 30 mm. thick. Many small cysts filled with a hemorrhagic fluid and many nodules, ranging in size to that of a fist, were seen. The pulmonary parenchyma was not involved. Histologic sections showed a great variety: accumulations of epithelial-like cells, tubular structures lined with low cuboidal cells, some areas resembling mixed tumors of the salivary glands, others of a fibrous, of a fibrosarcomatous and of an angiomatous character, and accumulations of tumor giant cells. A detailed analysis of the varied histologic features of the reported cases is given, as well as a discussion of the hypotheses dealing with the histogenesis of the pleural lining. The histologic polymorphism of most primary tumors of the pleura is in accord with Maximow's concept of the multiple potentialities of the pleural mesothelium.

I. DAVIDSOHN.

GANGLION CELL TUMORS OF THE BRAIN. E. CHRISTENSEN, *Virchows Arch. f. path. Anat.* **300**:567, 1937.

Thirty-seven tumors of the brain containing ganglion cells are tabulated and briefly reviewed. The varying nomenclature of these tumors, depending on the degree of differentiation, is discussed. The author describes 5 of the tumors. Four revealed a considerable degree of differentiation and were considered histologically benign. The fifth was believed to have arisen on the basis of congenital dysplasia. Most of the tumors arose in children and young adults. In 30 per cent of the series the growth occurred in the floor of the third ventricle. Because of the histologically benign character of many of these tumors the postoperative prognosis should be good if such a tumor is so situated that it can be completely removed.

O. T. SCHULTZ.

HISTOGENESIS OF THE SHOPE INOCULABLE RABBIT PAPILLOMA. P. LADEWIG and S. OBERNDORFER, *Virchows Arch. f. path. Anat.* **301**:204, 1938.

The histogenesis of the inoculable rabbit papilloma described by Shope in 1933 was studied in inoculated areas of skin removed at intervals of from one to three hundred and thirty-four days after inoculation. On the fifth day there begin to appear within the epidermis small areas, termed primary centers, each composed of a small number of swollen, faintly stained epithelial cells. These cells are not capable of division. Such centers continue to increase in number from the sixth to the ninth day, and during this period proliferation of the malpighian layer becomes evident. In time the barrier between epithelium and connective tissue is broken down, and a "precancerous" stage develops. The various types of epithelial cells composing the papilloma are derived from similar cells of the normal epidermis. The various cell inclusions observed are not characteristic of the rabbit papilloma but are seen in dyskeratotic lesions of the human skin. They are derived from substances formed within the epithelial cells. Elementary virus bodies have not been seen.

O. T. SCHULTZ.

THE NEVUS CELL. F. FEYRTER, *Virchows Arch. f. path. Anat.* **301**:417, 1938.

Although Soldan described the origin of cellular nevi from the terminal nerves of the skin in 1899, the neurogenic origin of nevi did not receive much attention until the much more recent work of Masson. Soldan derived the nevi from the connective tissue of the terminal nerves, whereas Masson derived them from the cells composing the sheath of Schwann and considered the nevus cell a special type of cell. Feyrter presents a monographic study of the nevus cell, based largely on the use of a metachromatic toluidine blue-staining method, which he has previously described. The nevus cells contain lipid substances of various kinds in the form of fine droplets, large vacuoles or crystals. At the periphery of the cell the material may become fibrillated, the fibrils becoming continuous as precollagen with the intercellular fibrils. Lamination of the cells leads to the formation of bodies like the Meissner tactile corpuscles described by Masson. Feyrter claims to have demonstrated lipid-containing cells in the epidermis (the intraepidermal pale nevus cells of Masson), at the junction of the epidermis with the cutis and in the cutis in the absence of nevus. It is their function to elaborate, store and utilize lipoids. They are part of a system which in the nerve fibers is the endoneurium and in the skin may give rise to the isolated cells described. These cells of the endoneurium Feyrter terms endothelium, and it is from them that the nevus cell is derived. The nevus, therefore, is a neurogenic endothelioma or neuro-endothelioma.

O. T. SCHULTZ.

Medicolegal Pathology

CHEMICAL TEST OF THE BREATH FOR INTOXICATION. R. N. HARGER, E. B. LAMB and H. R. HULPIEU, *J. A. M. A.* **110**:779, 1938.

A reagent for alcohol in the breath consists of 55 per cent sulfuric acid containing a measured small amount of permanganate solution. The permanganate reacts rapidly and quantitatively with alcohol at ordinary temperatures and is not affected by acetone.

The ratio of alcohol to carbon dioxide in the breath may be used to measure the concentration of alcohol in the blood. The weight of the alcohol accompanying 190 mg. of carbon dioxide in the breath is very nearly equal to the weight of the alcohol in 1 cc. of the subject's blood.

Employment of the ratio of alcohol to carbon dioxide in the breath permits the test to be made without the subject's being touched. A tube is held in the breath stream, and a pump draws the sample through the apparatus.

Tests made on 121 subjects showed a good correlation between the concentration of alcohol in 1 cc. of the blood and the amount of alcohol accompanying 190 mg. of carbon dioxide in the breath.

As collected in our tests, 4 liters of expired air contained about the same amount of alcohol as 1 cc. of the subject's blood. Because of possible fluctuations in the amount of alveolar air in such samples, it is believed that analyses of breath made on the volume basis should be checked by determining the carbon dioxide in the sample.

FROM AUTHORS' SUMMARY.

MEDICOLEGAL SIGNIFICANCE OF PRESSURE ON THE BRAIN. W. NEUGEBAUER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **29**:272, 1938.

Pressure on the brain is an expression of imbalance between the intracranial space and its solid and liquid elements. The pressure may be acute or chronic, latent or manifest. Usually the brain with its enveloping membranes is from 10 to 14 per cent smaller than the cranial cavity. Acute pressure is divided into two types: that due to edema of the brain and that due to swelling of the brain. The latter is difficult to recognize in children, but the brain is definitely more dense than when the pressure is due to edema. Acute pressure is brought about by trauma, including birth trauma, and by subdural and epidural hemorrhage. It may occur after the action of toxins; thus it is found after burns, in gastrointestinal catarrh in children, in rickets, and in uremic and diabetic coma. Exogenous toxins, such as alcohol, ether and chloroform, and poisons, such as lysol (compound solution of cresol), may also cause it. It is frequently seen in infections such as cerebrospinal meningitis.

Chronic pressure is manifest in alterations of the skull, among which are deep furrows for the arteries and thinning of the sella turcica. The dura often is thickened and has coarse pacchionian granulations. The leptomeninges may be edematous and at times calcified. Prolonged pressure may cause synostosis of the sutures. The chronic alterations associated with tumors are well known.

GEORGE J. RUKSTINAT.

INJURIES OF THE CERVICAL SPINAL CORD IN NEWBORN CHILDREN. F. HAUSBRANDT, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **29**:353, 1938.

Hausbrandt believes that at times minor alterations in the cervical portion of the spinal cord are regarded by pathologists as a cause of death, although, practically, they have no significance. Not every tear of the tentorium leads to death, as such tears have been encountered in children who survived such tears up to twenty days and then died of intercurrent infections. This is especially true of premature infants. When, on the other hand, the physiologic tension of the tentorium is altered because of the site of tears in it, pressure on the head in the birth canal is applied to the medulla oblongata so that the stimulus to respiration is initiated. The same pressure effects may be at work where there is massive hemorrhage in the posterior cranial fossa. The term "aspiration of amniotic fluid" does not indicate either a definite process or a clearcut diagnosis.

After a detailed technical consideration of the method to be employed in removing the spinal cord, the author discusses the pathologic changes he has encountered. Hemorrhage was found in the epidural space of the cervical region in some of the bodies examined, but it was not a regular accompaniment of intracranial hemorrhage. Usually such hemorrhages were associated with extensive injuries of the cervical part of the spinal cord; they occupied the upper portion and showed gradation from a bloody edema to massive, sleeve-like hemorrhages. Subdural hemorrhages were less frequent and extensive than epidural ones and usually were continuations of infratentorial extravasations. Hemorrhages in the meninges were relatively rare. Injury of the cord near its junction with the medulla was seen very frequently in full term stillborn children and in those dying shortly after birth. Regions of softening and hemorrhage were found most often at the junction of gray and white matter. The white substance was usually spared, but isolated or clumped hemorrhages were common in the region of the posterior horn.

GEORGE J. RUKSTINAT.

Society Transactions

NEW ENGLAND PATHOLOGICAL SOCIETY

CHARLES BRANCH, *President*

Regular Meeting, Feb. 16, 1939

GRANVILLE A. BENNETT, *Secretary*

CORRELATIONS BETWEEN POSTMORTEM TELEROENTGENOGRAMS OF THE CHEST AND AUTOPSY REPORTS, WITH SPECIAL REFERENCE TO PULMONARY EMBOLISM AND INFARCTION. BENJAMIN CASTLEMAN and (by invitation) AUBREY O. HAMPTON.

Accurate correlations between roentgenographic and autopsy observations are usually impossible because of the pathologic changes that frequently occur between the taking of good roentgenograms and death, the unsatisfactory quality of antemortem roentgenograms on films which of necessity are often portable and because of the collapsed state of the lungs post mortem.

In order to avoid these difficulties we are now taking anteroposterior and lateral postmortem roentgenograms of the chest at a distance of 7 feet (2 meters), with the subject upright. At autopsy the lungs, instead of being sectioned in their fresh state, are distended to approximately their inspiratory size by pouring solution of formaldehyde U. S. P. into the trachea. The trachea is then tied and the entire preparation put in solution of formaldehyde. One week later the lungs are sectioned in the presence of the roentgenologist, and an attempt is made to account for every shadow on the roentgenograms. Lungs from 400 persons have been examined in this way.

In 3,500 routine autopsies, 9 per cent showed embolism or infarction of the lung, and in 3.5 per cent embolism was the cause of death. In this series of 400 autopsies 14 per cent showed embolism or infarction—an increase of 50 per cent. Molds were made of many of the infarcts, and none showed the traditional triangular shape. Frequently an infarct in the costophrenic angle showed a convexity toward the hilus. Every infarct observed was peripheral, extending to the pleural surface of the lung. More frequently infarction occurred in areas where two or more pleural surfaces met.

From this study we have been able to obtain a fairly clear picture of the successive pulmonary changes that develop when an embolus reaches the lung. During the first day the infarcted lung still contains a good deal of air in the alveoli, and there is no sharp line of demarcation between infarcted and normal lung, nor is there any destruction of alveolar walls. Some air-containing alveoli are still present on the third day, but at this time a sharp line of demarcation is present, and red blood cells and white blood cells are found in the pulmonary alveoli and in their walls. Still later, the infarcted areas become encapsulated, and there is almost complete necrosis of the alveolar walls. Complete healing is evidenced by an organized fibrous scar, which shows as a linear shadow on the roentgenogram. Pathologists have been too lax in searching for or recognizing these healed infarcts, probably because the infarcts are difficult to find when the lungs are deflated.

One of the most important aspects of this work is the development of the concept of incomplete infarction. The term "incomplete infarction" is used to indicate an infarct in which the alveoli are partially filled with air, edema fluid, red cells and a few leukocytes but in which there is no destruction of the alveolar wall. These lesions do not organize like true infarcts but resolve in a few days.

They are seen most commonly in postpartum or postoperative patients. Temporarily, however, the roentgenologic and even the gross postmortem appearances may be indistinguishable from true early infarction. This condition occurs in a previously normal lung (if it took place in a congested lung, a true infarct would develop) and corresponds to the experimental lesions produced in normal dogs by such workers as Cohnheim, Litten and Karsner. These investigators considered such lesions as negative results, since there was no destruction of the alveolar wall. Clinical application of these negative results has not been made previously.

CHRONIC BENZENE POISONING. FRANCIS T. HUNTER (by invitation).

The report concerns clinical and hematologic studies made on 80 workers exposed to fumes of benzene, including 8 with clinical poisoning. Only 30 per cent of the 80 workers were thought to show no effects of the solvent. In the whole group, however, polycythemia occurred in 17.5 per cent and anemia in 29 per cent. In 12 workers (15 per cent) unexplained leukocytosis was present without other changes in the blood. A single patient had leukopenia without other changes.

The descending order of frequency of variations from the normal peripheral blood picture was leukocytosis, 34 per cent; decrease in the percentage of polymorphonuclears, 31 per cent; anemia, 29 per cent; presence of young polymorphonuclears with or without marrow cells, 28 per cent; absolute decrease in the number of polymorphonuclears, 26 per cent; eosinophilia, 25 per cent; polycythemia, 17.5 per cent; absolute increase in the number of polymorphonuclears, 17.5 per cent; leukopenia, 15 per cent; increased percentage of polymorphonuclears 5 per cent.

It is concluded that leukopenia is a poor index as to the effect of benzene on the marrow. In 10 of 12 instances in this series, when leukopenia was noted anemia was already present. Leukocytosis, a decreased percentage of polymorphonuclears, the presence of young polymorphonuclears or marrow cells, or eosinophilia is observable prior to the establishment of anemia. In some cases polycythemia may be the earliest detectable finding.

In 3 fatal cases the symptoms of poisoning appeared months after removal from exposure and seemed to be precipitated by the onset of an otherwise benign infection. This suggests that the mildly poisoned marrow may be adequate for normal demands but becomes decompensated under the added burden of infection.

HISTOLOGIC STUDIES OF CHRONIC BENZENE POISONING. E. A. GALL and TRACY B. MALLORY.

Histologic material from 14 persons with chronic benzene poisoning has been studied. Twelve of these persons were given complete autopsies, and 2, sternal biopsies.

The literature conveys the impression that benzene characteristically produces aplasia of the bone marrow, and when any attention has been directed to the extramedullary viscera the comments have implied that changes in these organs are unusual and adventitious.

Of the 12 persons examined post mortem, all showed evidence of primary benzene effects on the bone marrow, spleen, liver and lymph nodes. Bone marrow was hypoplastic in only 5, and in none was there total aplasia. Early changes consisted in marked increase of phagocytic activity, hemosiderosis and progressive fibrosis. Following a transitory increase in marrow fat, hemopoietic regeneration, almost wholly erythrogenic, developed. At first the regenerated cells were predominantly normoblasts, but later large numbers of "megaloblasts" and megakaryocyte-like cells appeared. These were associated with numerous mitotic figures.

The splenic and lymph node response likewise showed increased phagocytic activity and progressive fibrosis. Lymphoid elements were at first diminished, but they rapidly regenerated. Such regeneration was bizarre, however, and was

accompanied occasionally in the lymph nodes and frequently in the spleen by well defined hemopoiesis. With increasing chronicity there eventually appeared primitive red cells and multinucleated giant cells in such enormous numbers as to obscure the normal architecture.

Changes in the liver consisted only in marked phagocytic activity and relatively minimal megaloblastic and megakarocytic hemopoiesis.

The intense cellularity noted in late stages of this process as observed in bone marrow, spleen and lymph nodes, associated with rapidity of growth, immaturity of component cells, fibrosis and multinucleated giant cells simulating megakaryocytes, produced a superficial similarity to Hodgkin's sarcoma. No evidence of metastatic propensity or of involvement of organs not related to the reticuloendothelial system was, however, observed.

Book Reviews

Animal Pathology. Russell A. Runnells, D.V.M., M.S., Associate Professor of Veterinary Pathology, Iowa State College. Price, \$6. Pp. 464, with 127 illustrations. Ames, Iowa: Collegiate Press, Inc., 1938.

This is a textbook for elementary courses in pathology given in veterinary schools, usually in the second year of the curriculum. It is intended to serve as an introduction to the study of clinical veterinary medicine. Consequently it deals with animal pathology in its relations to current veterinary practice. It does not aim at comprehensive consideration of animal pathology in general. It is divided into three parts. The first part gives in condensed form a good summary of general pathology, that is "the general pathologic conditions which may occur in more than one tissue or organ." The second part deals with systemic pathology and the third with the special pathology of the various infectious diseases. These two parts are the author's book "A Guide to the Study of Special Veterinary Pathology" in revised and enlarged form. The illustrations are almost without exception instructive if not always artistic; many are taken from the *Journal of the American Veterinary Medical Association*. At the end of each chapter is a short list of appropriate references. The book will serve usefully as an introductory guide to the study of veterinary medicine.

Surgical Pathology. William Boyd, M.D., LL.D., M.R.C.P. (Edinburgh), F.R.C.P. (London), Dipl. Psych., F.R.C.S., Professor of Pathology, University of Toronto. Fourth edition, revised. Price, \$10. Pp. 886, with 491 illustrations. Philadelphia and London: W. B. Saunders Company, 1938.

Inguinal lymphogranuloma, grading of carcinoma, glomangioma, parathyroid tumor, autolytic peritonitis, arrhenoblastoma, certain ovarian tumors, regional ileitis and other new topics receive brief consideration, but nothing is said about the "surgical pathology" of the larynx, lungs, pericardium and heart. Certainly the advances and activities in the field of thoracic surgery should be reflected in a book on "surgical pathology." New material has been introduced in the chapters on cancer, thrombosis, gastric ulcer, puerperal sepsis and appendicitis. The chapter on the surgeon and the laboratory is long out of date.

Etudes sur la rage. P. Remlinger, directeur de l'Institut Pasteur de Tanger, and J. Bailly, chef de service a l'Institut Pasteur de Tanger. Price, 40 francs. Pp. 174. Paris: Masson & Cie, 1938.

P. Remlinger was director of the Institut antirabique de Constantinople from 1903 to 1910. In an appendix are listed the papers published by him on rabies during that period. Several years later he became director of the Pasteur Institute of Tangiers, Morocco, S. Africa, and from 1917 to 1937, inclusive, he published as a single or as a joint author 201 papers on the results of scientific studies of rabies. The papers are listed in chronologic order. The book gives concise summaries of these contributions from the Tangiers period, which concern many various phases of rabies in man and animals. The book will interest especially the scientific student of rabies.

Books Received

TROPANOL ET PSEUDOTROPANOL. ACTIONS PHYSIOLOGIQUES COMPARÉES. René Hazard, Professeur à la Faculté de Médecine de Paris. Paper. Pp. 88, with 24 illustrations. Price 25 francs. Paris: Masson & Cie, 1939.

LA MORT DES BRULÉS. ETUDE EXPÉRIMENTALE. Louis Christophe, Chargé de cours à l'Université de Liège. Preface by Professeur Léon Binet. Paper. Pp. 84, with 19 illustrations. Price, 40 francs. Paris: Masson & Cie, 1939.

CLINIQUE ET PATHOLOGIE COMPARÉES: VÉNÉRÉOLOGIE, CANCÉROLOGIE, DERMATOSES, MÉDECINE GÉNÉRALE, PHYTOPATHOLOGIE. Louis Bory, Chef de clinique à l'Hôpital Saint-Louis. Preface by Professeur M. Fiessinger. Paper. Pp. 240. Price 50 francs. Paris: Masson & Cie, 1939.

THE FIFTY-FOURTH ANNUAL MEDICAL REPORT OF THE TRUDEAU SANATORIUM AND THE THIRTY-FOURTH MEDICAL SUPPLEMENT FOR THE YEAR ENDING SEPTEMBER 30, 1938, TOGETHER WITH THE TWENTY-SECOND COLLECTION OF THE STUDIES OF THE EDWARD L. TRUDEAU FOUNDATION FOR RESEARCH AND TEACHING IN TUBERCULOSIS, 1938.

THE SARANAC LABORATORY FOR THE STUDY OF TUBERCULOSIS OF THE EDWARD L. TRUDEAU FOUNDATION: REPORT OF THE DIRECTOR AND FINANCIAL REPORT FOR THE YEAR ENDING SEPTEMBER 30, 1938. REPRINTS OF SCIENTIFIC PAPERS. Saranac Lake, N. Y.: The Saranac Lake Academy of Medicine, 1938.

INTRACRANIAL TUMORS OF INFANCY AND CHILDHOOD. Percival Bailey, Douglas N. Buchanan and Paul C. Bucy, University of Chicago Clinics. Cloth. Pp. 598, with 113 illustrations. Price \$5. Chicago: University of Chicago Press, 1939.

CLASSIFIED AND ANNOTATED BIBLIOGRAPHY OF SIR WILLIAM OSLER'S PUBLICATIONS (BASED ON THE CHRONOLOGICAL BIBLIOGRAPHY OF MINNIE WRIGHT BLOGG). Edited by Maude E. Abbott, B.A., M.D., L.L.D. (McGill). Second edition, revised and indexed. Pp. 163. Price \$2.25. Montreal: 1939.

Reprinted with additions from the Sir William Osler Memorial Volume of the International Association of Medical Museums (Bulletin IX, 1926, pp. 437-605).

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Volume 27
1939

PUBLISHERS
AMERICAN MEDICAL ASSOCIATION
CHICAGO, ILL.

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JUNE 1939

PUBLISHED MONTHLY BY AMERICAN MEDICAL ASSOCIATION, 535 NORTH
DEARBORN STREET, CHICAGO, ILLINOIS. ANNUAL SUBSCRIPTION, \$6.00

Entered as Second Class Matter Jan. 20, 1926, at the Postoffice at Chicago,
Under the Act of Congress of March 3, 1879

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